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(54) Title: PHOSPHATIDYL INOSITOL 3-KINASE DELTA BINDING PARTNER

(57) Abstract: There is identified a functional interaction between the catalytic subunit of phosphatidyl inositol 3-kinase delta (P13K $\delta$ ) and SH3 domain-containing polypeptides such as LASP-1. The invention provides methods of assaying the observed interaction, methods of exploiting the interaction to identify compounds that modulate the interaction, and methods of employing such modulators in the treatment of medical disorders characterized by P13K $\delta$  activity mediated by the interaction.

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**WO 01/85986 A2**

## PHOSPHATIDYL INOSITOL 3-KINASE DELTA BINDING PARTNER

The present invention relates generally to phosphatidylinositol 3-kinase delta (PI3K $\delta$ ) enzyme, and more particularly to binding partners of PI3K $\delta$ , and to methods of using such materials.

### BACKGROUND OF THE INVENTION

Cell signaling via 3'-phosphorylated phosphoinositides has been implicated in a variety of cellular processes, e.g., malignant transformation, growth factor signaling, inflammation, and immunity [see Rameh et al., *J Biol Chem* 274:8347-8350 (1999) for a review]. The enzyme responsible for generating these phosphorylated signaling products, phosphatidylinositol 3-kinase (PI 3-kinase; PI3K), was originally identified as an activity associated with viral oncoproteins and growth factor receptor tyrosine kinases that phosphorylates phosphatidylinositol (PI) and its phosphorylated derivatives at the 3'-hydroxyl of the inositol ring [Panayotou et al., *Trends Cell Biol* 2:358-60 (1992)].

The levels of phosphatidylinositol-3,4,5-triphosphate (PIP<sub>3</sub>), the primary product of PI 3-kinase activation, increase upon treatment of cells with a variety of agonists. PI 3-kinase activation is, therefore, believed to be involved in a range of cellular responses including cell growth, differentiation, and apoptosis [Parker et al., *Current Biology* 5:577-99 (1995); Yao et al., *Science* 267:2003-05 (1995)]. Though the downstream targets of phosphorylated lipids generated following PI 3-kinase activation have not been well characterized, emerging evidence suggests that pleckstrin-homology domain- and FYVE-finger domain-containing proteins are activated upon binding to various phosphatidylinositol lipids [Sternmark et al., *J Cell Sci* 112:4175-83 (1999); Lemmon et al., *Trends Cell Biol* 7:237-42 (1997)]. *In vitro*, some isoforms of protein kinase C (PKC) are directly activated by PIP<sub>3</sub>, and the PKC-related protein kinase, PKB, has been shown to be activated by PI 3-kinase [Burgering et al., *Nature* 376:599-602 (1995)].

Presently, the PI 3-kinase enzyme family has been divided into three classes based on their substrate specificities. Class I PI3Ks can phosphorylate phosphatidylinositol (PI), phosphatidylinositol-4-phosphate, and phosphatidylinositol-4,5-biphosphate (PIP<sub>2</sub>) to produce phosphatidylinositol-3-phosphate (PIP), phosphatidylinositol-3,4,5-triphosphate (PIP<sub>3</sub>), and phosphatidylinositol-3-phosphate (PIP<sub>2</sub>).

inositol-3,4-bisphosphate, and phosphatidyl inositol-3,4,5-triphosphate, respectively. Class II PI3Ks phosphorylate PI and phosphatidyl inositol-4-phosphate, whereas Class III PI3Ks can only phosphorylate PI.

The initial purification and molecular cloning of PI 3-kinase revealed that it was a heterodimer consisting of p85 and p110 subunits [Otsu et al., *Cell* 65:91-104 (1991); Hiles et al., *Cell* 70:419-29 (1992)]. Since then, four distinct Class I PI3Ks have been identified, designated PI3K  $\alpha$ ,  $\beta$ ,  $\delta$ , and  $\gamma$ , each consisting of a distinct 110 kDa catalytic subunit and a regulatory subunit. More specifically, three of the catalytic subunits, i.e., p110 $\alpha$ , p110 $\beta$  and p110 $\delta$ , each interact with the same regulatory subunit, p85; whereas p110 $\gamma$  interacts with a distinct regulatory subunit, p101. As described below, the patterns of expression of each of these PI3Ks in human cells and tissues are also distinct. Though a wealth of information has been accumulated in recent past on the cellular functions of PI 3-kinases in general, the roles played by the individual isoforms are largely unknown.

Cloning of bovine p110 $\alpha$  was described in Hiles et al. [*supra*]. This protein was identified as related to the *Saccharomyces cerevisiae* protein: Vps34p, a protein involved in vacuolar protein processing [*Id.*]. The recombinant p110 $\alpha$  product was also shown to associate with p85 $\alpha$ , to yield a PI3K activity in transfected COS-1 cells [Hiles et al., *supra*].

The cloning of a second human p110 isoform, designated p110 $\beta$ , is described by Hu et al. [*Mol Cell Biol* 13:7677-88 (1993)]. This isoform is said to associate with p85 in cells, and to be ubiquitously expressed, as p110 $\beta$  mRNA has been found in numerous human and mouse tissues as well as in human umbilical vein endothelial cells, Jurkat human leukemic T cells, 293 human embryonic kidney cells, mouse 3T3 fibroblasts, HeLa cells, and NBT2 rat bladder carcinoma cells [*Id.*]. Such wide expression suggests that this isoform is broadly important in signaling pathways.

Identification of the p110 $\delta$  isoform of PI 3-kinase is described in Chantray et al., *J Biol Chem* 272:19236-41 (1997). It was observed that the human p110 $\delta$  isoform is expressed in a tissue-restricted fashion; it is expressed at high levels in lymphocytes and lymphoid tissues, suggesting that the protein might play a role in PI 3-kinase-mediated signaling in the immune system. Details concerning the P110 $\delta$  isoform may also be found in U.S. Patent Nos. 5,858,753, 5,822,910, and 5,985,589.

See also, Vanhaesebroeck et al., *Proc Natl Acad Sci USA* 94:4330-5 (1997), and international publication WO 97/46688.

In each of the PI3K $\alpha$ ,  $\beta$ , and  $\delta$  subtypes, the p85 subunit acts to localize PI 3-kinase to the plasma membrane by the interaction of its SH2 domain with phosphorylated tyrosine residues (present in an appropriate sequence context) in target proteins [Rameh et al., *Cell* 83:821-30 (1995)]. Two isoforms of p85 have been identified, p85 $\alpha$ , which is ubiquitously expressed, and p85 $\beta$ , which is primarily found in the brain and lymphoid tissues [Volinia et al., *Oncogene* 7:789-93 (1992)]. Association of the p85 subunit to the PI 3-kinase p110 $\alpha$ ,  $\beta$ , or  $\delta$  catalytic subunits appears to be required for the catalytic activity and stability of these enzymes. In addition, the binding of Ras proteins also upregulates PI 3-kinase activity.

The cloning of p110 $\gamma$  revealed still further complexity within the PI3K family of enzymes [Stoyanov et al., *Science* 269:690-93 (1995)]. The p110 $\gamma$  isoform is closely related to p110 $\alpha$  and p110 $\beta$  (45-48% identity in the catalytic domain), but as noted does not make use of p85 as a targeting subunit. Instead, p110 $\gamma$  contains an additional domain termed a "pleckstrin homology domain" near its amino terminus. This domain allows interaction of p110 $\gamma$  with the  $\beta\gamma$  subunits of heterotrimeric G proteins and this interaction appears to regulate its activity [Stoyanov et al., *supra*].

The p101 regulatory subunit for PI3K $\gamma$  was originally cloned in swine, and the human ortholog identified subsequently [Krugmann et al., *J Biol Chem* 274:17152-8 (1999)]. Interaction between the N-terminal region of p101 with the N-terminal region of p110 $\gamma$  appears to be critical for the PI3K $\gamma$  activation through G $\beta\gamma$  mentioned above.

A constitutively active PI3K polypeptide is described in international publication WO 96/25488. This document describes preparation of a chimeric fusion protein in which a 102-residue fragment of p85 known as the inter-SH2 (iSH2) region is fused through a linker region to the N-terminus of murine p110. The p85 iSH2 domain apparently is able to activate PI3K activity in a manner comparable to intact p85 [Klippel et al., *Mol Cell Biol* 14:2675-85 (1994)].

Thus, PI 3-kinases may be defined by their amino acid identity or by their activity. Additional members of this growing gene family include more distantly related lipid and protein kinases including Vps34 TOR1, and TOR2 of



*Saccharomyces cerevisiae* (and their mammalian homologs such as FRAP and mTOR), the ataxia telangiectasia gene product (ATR) and the catalytic subunit of DNA-dependent protein kinase (DNA-PK). See generally, Hunter, *Cell* 83:1-4 (1995).

5 PI 3-kinase also appears to be involved in a number of aspects of leukocyte activation. A p85-associated PI 3-kinase activity has been shown to physically associate with the cytoplasmic domain of CD28, which is an important costimulatory molecule for the activation of T-cells in response to antigen [Pages et al., *Nature* 369:327-29 (1994); Rudd, *Immunity* 4:527-34 (1996)]. Activation of T cells through  
10 CD28 lowers the threshold for activation by antigen and increases the magnitude and duration of the proliferative response. These effects are linked to increases in the transcription of a number of genes including interleukin-2 (IL2), an important T cell growth factor [Fraser et al., *Science* 251:313-16 (1991)]. Mutation of CD28 such that it can no longer interact with PI 3-kinase leads to a failure to initiate IL2 production,  
15 suggesting a critical role for PI 3-kinase in T cell activation [Pages et al., *supra*].

Specific inhibitors against individual members of a family of enzymes provide invaluable tools for deciphering function(s) of each enzyme. Two compounds, LY294002 and wortmannin, have been widely used as PI 3-kinase inhibitors. These compounds, however, are non-specific PI3K inhibitors, as they do not distinguish  
20 among the four members of Class I PI 3-kinases. For example, the  $IC_{50}$ 's of wortmannin against each of the various Class I PI 3-kinases are in the range of 1-10 nM. Similarly, the  $IC_{50}$  for LY294002 against each of these PI 3-kinases is about 1  $\mu$ M [Fruman et al., *Ann Rev Biochem* 67:481-507 (1998)]. Hence, the utility of these compounds in studying the roles of individual Class I PI 3-kinases is limited.

25 Based on studies using the wortmannin, there is evidence that PI 3-kinase function is also required for some aspects of leukocyte signaling through G-protein coupled receptors [Thelen et al., *Proc Natl Acad Sci USA* 91:4960-64 (1994)]. Moreover, it has been shown that wortmannin and LY294002 block neutrophil migration and superoxide release. However, inasmuch as these compounds do not  
30 distinguish among the various isoforms of PI3K, it remains unclear which particular PI3K isoform or isoforms are involved in these phenomena.

In view of the above considerations, it is clear that existing knowledge is lacking with respect to structural and functional features of the PI 3-kinase enzymes, including sub-cellular localization, activation states, substrate affinities, and the like. Moreover, the functions that these enzymes perform in both normal and diseased tissues remains to be elucidated. In particular, the function of PI3K $\delta$  in leukocytes has not previously been characterized, and knowledge concerning its function in human physiology remains limited. The coexpression in these tissues of other PI3K isoforms has heretofore confounded efforts to segregate the activities of each enzyme. Furthermore, separation of the activities of the various PI3K isozymes may not be possible without identification of inhibitors that demonstrate selective inhibition characteristics.

Thus, there exists a need in the art for further structural and functional characterization of the PI3K $\delta$  enzyme. Furthermore, our understanding of PI3K $\delta$  requires further elaboration of the structural interactions of p110 $\delta$ , both with its regulatory subunit and with other proteins in the cell. There also remains a need for selective or specific inhibitors of PI3K isozymes, in order that the functions and structural interactions of each isozyme may be better characterized.

One of the purposes of the present invention is to provide methods for identifying compounds that can inhibit PI3K $\delta$  activity, and preferably compounds that inhibit interaction of PI3K $\delta$  with its binding partners. Another purpose of the invention is to provide compounds that inhibit PI3K $\delta$  selectively while having relatively low inhibitory potency against the other PI3K isoforms. Another purpose of the invention is to provide methods of characterizing the function of human PI3K $\delta$ . Another purpose of the invention is to provide methods of selectively modulating human PI3K $\delta$  activity, and thereby to promote medical treatment of diseases mediated by PI3K $\delta$  dysfunction. Other purposes and advantages of the invention will be readily apparent to the artisan having ordinary skill in the art.

## SUMMARY OF THE INVENTION

It has now been discovered that these and other purposes can be achieved by the present invention, which, in one aspect, is a method of identifying a modulator of p110 $\delta$  binding to LASP-1, comprising the steps of:

- 5 (a) providing a p110 $\delta$  polypeptide and a LASP-1 polypeptide having specific binding affinity for one another;
- (b) measuring binding between the p110 $\delta$  polypeptide and the LASP-1 polypeptide in the presence and absence of a test compound; and
- (c) identifying the test compound as a modulator of p110 $\delta$  binding to  
10 LASP-1 when a different amount of binding of the p110 $\delta$  polypeptide to the LASP-1 polypeptide is detected in the presence of the test compound than in the absence of the test compound.

The p110 $\delta$  polypeptide and the LASP-1 polypeptide can be provided in a system free of cellular components, or may be recombinantly expressed or  
15 coexpressed in host cells. In some embodiments, the p110 $\delta$  polypeptide comprises the proline rich region of p110 $\delta$ . Likewise, in some embodiments, the LASP-1 polypeptide comprises the SH3 region of LASP-1. Either or both of the polypeptides may be provided as fusion proteins to impart desirable properties to the polypeptides, e.g., to permit secretion or cell surface expression of the polypeptides. The method  
20 may be employed to identify modulators that inhibit binding of p110 $\delta$  to LASP-1 or modulators that enhance binding of p110 $\delta$  to LASP-1.

In another aspect, the invention is a compound having activity as a modulator of interaction between p110 $\delta$  and LASP-1, wherein the compound is identified according to the method described herein. For example, the compound may be a  
25 compound that inhibits binding of p110 $\delta$  to LASP-1, or a compound that enhances binding of p110 $\delta$  to LASP-1.

In another aspect, the invention is a method of treating a disease state characterized by undesirable or excessive activity of PI3K $\delta$ , comprising administering to a subject in need thereof a compound that modulates interaction of  
30 p110 $\delta$  with LASP-1 in an amount effective to modulate interaction of p110 $\delta$  with LASP-1. Preferably, the compound inhibits p110 $\delta$ :LASP-1 interaction. Compounds

suitable for use in this method may be identified using screening methods described herein, or may be derived analogs thereof.

In still another aspect, the invention is a method of modulating p110 $\delta$  binding to LASP-1, comprising the step of contacting p110 $\delta$  or LASP-1 with a modulator of p110 $\delta$  binding to LASP-1. Preferably, the method employs a modulator that inhibits binding of p110 $\delta$  to LASP-1.

In yet another aspect, the invention is a method of treating a disease state associated with p110 $\delta$  binding to LASP-1, comprising the step of administering to a subject in need thereof an effective amount of a modulator of p110 $\delta$  binding to LASP-1. It is preferred that the method employs a modulator that inhibits binding of p110 $\delta$  to LASP-1.

As noted in the examples herein, LASP-1 comprises an SH3 domain that interacts with the proline rich sequence of p110 $\delta$  (aa 288-314 of SEQ ID NO: 2). This proline rich sequence is contemplated as a site of interaction with other polypeptides comprising one or more SH3 domains, for example, Src family kinases, adapter proteins vav and cbl, the p47-phox component of NADPH oxidase, Bruton's tyrosine kinase (Btk) and the p85 component of PI3 kinase. Therefore, in another aspect, the invention is a method of identifying a modulator of p110 $\delta$  binding to a polypeptide comprising an SH3 domain, comprising the steps of:

- (a) providing a p110 $\delta$  polypeptide and an SH3 domain-containing polypeptide having specific binding affinity for one another;
- (b) measuring binding between the p110 $\delta$  polypeptide and the SH3 domain-containing polypeptide in the presence and absence of a test compound; and
- (c) identifying the test compound as a modulator of p110 $\delta$  binding to the SH3-domain containing polypeptide when a different amount of binding of the p110 $\delta$  polypeptide to the SH3 domain-containing polypeptide is detected in the presence of the test compound than in the absence of the test compound.

The p110 $\delta$  polypeptide and the SH3 domain-containing polypeptide can be provided in a system free of cellular components, or may be recombinantly expressed or coexpressed in host cells. In these embodiments, the p110 $\delta$  polypeptide comprises the proline rich region of p110 $\delta$ . Either or both of the polypeptides may be provided

as fusion proteins to impart desirable properties to the polypeptides, e.g., to permit secretion or cell surface expression of the polypeptides. The method may be employed to identify modulators that inhibit binding of p110 $\delta$  to SH3 domain-containing polypeptides or modulators that enhance binding of p110 $\delta$  to SH3 domain-containing polypeptides.

In another aspect, the invention is a compound having activity as a modulator of interaction between p110 $\delta$  and an SH3 domain-containing polypeptide, wherein the compound is identified according to the method described herein. For example, the compound may be a compound that inhibits binding of p110 $\delta$  to an SH3 domain-containing polypeptide, or a compound that enhances binding of p110 $\delta$  to an SH3 domain-containing polypeptide.

In another aspect, the invention is a method of treating a disease state characterized by undesirable or excessive activity of PI3K $\delta$ , comprising administering to a subject in need thereof a compound that modulates interaction of p110 $\delta$  with an SH3 domain-containing polypeptide in an amount effective to modulate interaction of p110 $\delta$  with an SH3 domain-containing polypeptide. Preferably, the compound inhibits p110 $\delta$ :SH3 domain-containing polypeptide interaction. Compounds suitable for use in this method may be identified using screening methods described herein, or may be derived analogs thereof.

In still another aspect, the invention is a method of modulating p110 $\delta$  binding to an SH3 domain-containing polypeptide, comprising the step of contacting p110 $\delta$  or the SH3 domain-containing polypeptide with a modulator of p110 $\delta$  binding to the SH3 domain-containing polypeptide. Preferably, the method employs a modulator that inhibits binding of p110 $\delta$  to the SH3 domain-containing polypeptide.

In yet another aspect, the invention is a method of treating a disease state associated with p110 $\delta$  binding to an SH3 domain-containing polypeptide, comprising the step of administering to a subject in need thereof an effective amount of a modulator of p110 $\delta$  binding to the SH3 domain-containing polypeptide. It is preferred that the method employs a modulator that inhibits binding of p110 $\delta$  to the SH3 domain-containing polypeptide.

These and other features and advantages of the present invention will be appreciated from the detailed description and examples that are set forth herein. The detailed description and examples are provided to enhance the understanding of the invention, but are not intended to limit the scope of the invention. In particular, while the following detailed description and examples focus on the interaction of p110 $\delta$  and LASP-1, the detailed description and examples are intended to be illustrative of interaction of p110 $\delta$  with other polypeptides comprising SH3 domains. Thus, for example, in the detailed description below discussion of LASP-1 is applicable to SH3 domain-containing polypeptides such as Src family kinases, adapter proteins vav and cbl, the p47-phox component of NADPH oxidase, Bruton's tyrosine kinase (Btk) and the p85 component of PI3 kinase.

#### DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The invention provides methods of characterizing an interaction between the p110 $\delta$  subunit of phosphatidyl inositol 3-kinase delta (PI3K $\delta$ ), i.e., p110 $\delta$ , and LASP-1. There are further provided methods of identifying modulators, e.g., inhibitors and enhancers, of p110 $\delta$ :LASP-1 binding. Also provided are methods of employing modulators of PI3K $\delta$  activity associated with or mediated by p110 $\delta$ :LASP-1 binding, including methods of modulating the activity of the PI3K $\delta$  isozyme in cells, especially leukocytes, osteoclasts, and cancer cells. The methods include *in vitro*, *in vivo*, or *ex vivo* applications. Of particular benefit are methods of selectively modulating p110 $\delta$ :LASP-1 interactions in the clinical setting, to treat disease states mediated by PI3K $\delta$  activity. Thus, disease states characterized by excessive or inappropriate PI3K $\delta$  activity may be treated through use of selective modulators of p110 $\delta$ :LASP-1 binding according to the invention. Other methods enabled by the invention include methods necessary for further characterization of the p110 $\delta$ :LASP-1 interaction *in vivo*. Details of these and other useful embodiments of the invention are now described.

The methods described herein benefit from the use of compounds that selectively inhibit p110 $\delta$ :LASP-1 interaction in cells *in vitro*, *in vivo*, or *ex vivo*. Cells useful in the methods include those that express endogenous p110 $\delta$  and LASP-1. By "endogenous" it is meant that the cells express p110 $\delta$  and/or LASP-1 absent

recombinant introduction into the cells of one or more polynucleotides encoding the polypeptide(s) or biologically active fragments thereof. Methods also encompass use of host cells that have been modified recombinantly to express exogenous p110 $\delta$  and/or LASP-1, wherein one or more polynucleotides encoding p110 $\delta$  or LASP-1, or  
5 biologically active fragments thereof, have been introduced into the cell using recombinant procedures. Host cells coexpressing a p110 $\delta$  polypeptide and a LASP-1 polypeptide are provided, as are recombinant expression vectors enabling such coexpression, e.g., vectors comprising DNAs encoding the polypeptides arranged in tandem. Of particular advantage, the cells may be *in vivo*, i.e., in a living subject,  
10 e.g., an animal or human, wherein a modulator of p110 $\delta$ :LASP-1 binding may be used as a therapeutic to modulate PI3K $\delta$  activity in the subject. Alternatively, the cells may be isolated as discrete cells or in a tissue, for *ex vivo* or *in vitro* methods.

*In vitro* methods also comprehended by the invention can comprise the step of contacting an isolated p110 $\delta$  polypeptide or LASP-1 polypeptide with a modulator of  
15 p110 $\delta$ :LASP-1 binding. In such "biochemical" methods, the p110 $\delta$  and LASP-1 polypeptides are provided as purified and isolated polypeptides, i.e., the polypeptides are isolated from a natural source (e.g., cells or tissues that normally express at least one of the polypeptides absent modification by recombinant technology) or isolated from cells modified by recombinant techniques to express at least one of the  
20 polypeptides.

Compounds of any type that selectively modulate p110 $\delta$ :LASP-1 binding may be used as modulators in the methods of the invention. Moreover, compounds of any type that modulate, preferably inhibit, p110 $\delta$ :LASP-1 binding and that possess acceptable pharmacological properties may be used as modulators in the therapeutic  
25 methods of the invention.

The relative efficacies of compounds as modulators of a biological activity may be established by determining the concentrations at which each compound affects the activity to a predefined extent and then comparing the results. Typically, the preferred determination is the concentration that inhibits 50% of the activity in a  
30 biochemical assay, i.e., the 50% inhibitory concentration or "IC<sub>50</sub>." IC<sub>50</sub> determinations may be accomplished using conventional techniques known in the art. In general, IC<sub>50</sub> may be determined by measuring the given biological activity in the

presence of a range of concentrations of the inhibitor under study. The experimentally obtained values of enzyme activity are then plotted against the inhibitor concentrations used. The concentration of the inhibitor that shows 50% activity (as compared to the activity in the absence of any inhibitor) is taken as the IC<sub>50</sub>. Analogously, other inhibitory concentrations may be defined through appropriate determinations of activity. For example, in some settings it may be desirable to establish a 90% inhibitory concentration, i.e., IC<sub>90</sub>, etc.

#### Methods for Identifying Modulators of p110δ:LASP-1 Binding

The p110δ and LASP-1 polypeptides, as well as fragments thereof possessing biological activity, can be used for screening putative modulator compounds in any of a variety of drug screening techniques. A modulator of p110δ:LASP-1 binding is a compound that increases or decreases the ability of p110δ and LASP-1 to interact with one another, with the consequence of affecting the ability of p110δ to carry out any of its biological functions. An example of such a compound is an agent that, through affecting p110δ:LASP-1 binding, increases or decreases the ability of PI3Kδ to phosphorylate phosphatidyl inositol or to target to appropriate structures within a cell.

The selectivity of a compound that modulates p110δ:LASP-1 binding can be evaluated by comparing its activity on p110δ:LASP-1 binding to its activity on other related proteins or the interactions on other related binding pairs of proteins. To illustrate, selective modulators of p110δ:LASP-1 binding may include, for example, antibodies and other proteins or peptides that specifically bind to a p110δ polypeptide or a LASP-1 polypeptide, oligonucleotides that specifically bind to a p110δ polypeptide or a LASP-1 polypeptide, and other non-peptide compounds (e.g., isolated or synthetic organic molecules) that specifically interact with a p110δ polypeptide or a LASP-1 polypeptide.

Accordingly, the invention provides methods of characterizing the potency of a test compound as a modulator of p110δ:LASP-1 binding, said method comprising the steps of (a) measuring activity of a PI3Kδ polypeptide in the presence of a test compound; (b) comparing the activity of the PI3Kδ polypeptide in the presence of the



test compound to the activity of the PI3K $\delta$  polypeptide in the presence of an equivalent amount of a reference compound; wherein a lower activity of the PI3K $\delta$  polypeptide in the presence of the test compound than in the presence of the reference indicates that the test compound is a more potent inhibitor than the reference compound, and a higher activity of the PI3K $\delta$  polypeptide in the presence of the test compound than in the presence of the reference indicates that the test compound is a less potent inhibitor than the reference compound.

The invention further provides methods of characterizing the potency of a test compound as an inhibitor of p110 $\delta$ :LASP-1 binding, comprising the steps of (a) determining an amount of a control compound that inhibits p110 $\delta$ :LASP-1 binding by a reference percentage of inhibition, thereby defining a reference inhibitory amount for the control compound; (b) determining an amount of a test compound that inhibits p110 $\delta$ :LASP-1 binding by a reference percentage of inhibition, thereby defining a reference inhibitory amount for the test compound; (c) comparing the reference inhibitory amount for the test compound to the reference inhibitory amount for the control compound, wherein a lower reference inhibitory amount for the test compound than for the control compound indicates that the test compound is a more potent inhibitor than the control compound, and a higher reference inhibitory amount for the test compound than for the control compound indicates that the test compound is a less potent inhibitor than the control compound. In one aspect, the method uses a reference inhibitory amount that is the amount of the compound that inhibits p110 $\delta$ :LASP-1 binding by 50%, 60%, 70%, 80%. In another aspect, the method employs a reference inhibitory amount that is the amount of the compound that inhibits p110 $\delta$ :LASP-1 binding by 90%, 95%, or 99%. These methods may comprise determining the reference inhibitory amount of the compounds in an *in vitro* biochemical assay, in an *in vitro* cell-based assay, or in an *in vivo* assay.

The invention therefore provides a method for screening for candidate modulators of PI3K $\delta$  activity and/or to confirm the mode of action of candidate such negative regulators, i.e., to determine whether such compound operate through modulation of p110 $\delta$ :LASP-1 binding. Such methods may be employed against other p110 isoforms (e.g., p110 $\alpha$ , p110 $\beta$ , and p110 $\gamma$ ) in parallel to establish comparative activity of the test compound across the isoforms.

In these methods, the p110 $\delta$  polypeptide may be a full-length p110 $\delta$  polypeptide, e.g., human p110 $\delta$  having the sequence set forth in SEQ ID NO:2, or it may be a p110 $\delta$  fragment, provided that the fragment exhibits binding activity for LASP-1. An exemplary p110 $\delta$  polypeptide is a fragment of p110 $\delta$  comprising the proline-rich region that has been identified hereunder as interacting with LASP-1. The p110 $\delta$  fragment may further comprise the catalytic site of p110 $\delta$  and/or the p85 binding domain of p110 $\delta$ .

Likewise, the LASP-1 polypeptide may be a full-length LASP-1 polypeptide, e.g., human LASP-1 having the sequence set forth in SEQ ID NO:4, or it may be a LASP-1 fragment, provided that the fragment exhibits binding activity for p110 $\delta$ . An exemplary LASP-1 polypeptide is a fragment of LASP-1 comprising the SH3 region that has been identified hereunder as interacting with p110 $\delta$ . The LASP-1 polypeptide may further comprise other domains of the LASP-1 protein, e.g., the LIM region.

The methods may be employed in cells expressing cells expressing p110 $\delta$  or fragments thereof, either endogenously or exogenously. Accordingly, the polypeptide employed in such methods may be free in solution, affixed to a solid support, modified to be displayed on a cell surface (e.g., as a fusion protein), or located intracellularly. The modulation of activity or the formation of binding complexes between the p110 $\delta$  polypeptide, the LASP-1 polypeptide, and the agent being tested may then be measured.

LASP-1 and p110 $\delta$  polypeptides, and the interactions thereof, are amenable to biochemical or cell-based high throughput screening (HTS) assays according to methods known and practiced in the art, including melanophore assay systems to investigate receptor-ligand interactions, yeast-based assay systems, and mammalian cell expression systems. For a review, see Jayawickreme and Kost, *Curr Opin Biotechnol* 8:629-34 (1997). Automated and miniaturized HTS assays are also comprehended as described, for example, in Houston and Banks, *Curr Opin Biotechnol* 8:734-40 (1997).

Such HTS assays are used to screen libraries of compounds to identify particular compounds that exhibit a desired property. Any library of compounds may be used, including chemical libraries, natural product libraries, and combinatorial

libraries comprising random or designed oligopeptides, oligonucleotides, or other organic compounds.

Chemical libraries may contain known compounds, proprietary structural analogs of known compounds, or compounds that are identified from natural product screening.

Natural product libraries are collections of materials isolated from natural sources, typically, microorganisms, animals, plants, or marine organisms. Natural products are isolated from their sources by fermentation of microorganisms followed by isolation and extraction of the fermentation broths or by direct extraction from the microorganisms or tissues (plants or animal) themselves. Natural product libraries include polyketides, non-ribosomal peptides, and variants (including non-naturally occurring variants) thereof. For a review, see Cane et al., *Science* 282:63-68 (1998).

Combinatorial libraries are composed of large numbers of related compounds, such as peptides, oligonucleotides, or other organic compounds as a mixture. Such compounds are relatively straightforward to design and prepare by traditional automated synthesis protocols, PCR, cloning or proprietary synthetic methods. Of particular interest are peptide and oligonucleotide combinatorial libraries.

Still other libraries of interest include peptide, protein, peptidomimetic, multiparallel synthetic collection, recombinatorial, and polypeptide libraries. For a review of combinatorial chemistry and libraries created thereby, see Myers, *Curr Opin Biotechnol* 8:701-07 (1997).

5           Once compounds have been identified that show activity as modulators of p110δ:LASP-1 binding (i.e., "hits"), a program of optimization can be undertaken in an effort to improve the potency and/or selectivity of the activity. Analysis of structure-activity relationships (SAR) typically involves of iterative series of selective  
10       modifications of compound structures and their correlation to biochemical or biological activity. Families of related compounds can be designed that all exhibit the desired activity, with certain members of the family, namely those possessing suitable pharmacological profiles, potentially qualifying as therapeutic candidates. Compounds obtained using such medicinal chemistry methods may be referred to a "derived analogs" of the original "hit" compounds.

15           Related to methods of identifying modulators of p110δ:LASP-1 binding, the invention enables tools that facilitate such methods. For example, containers can be manufactured that contain a p110δ polypeptide and a LASP-1 polypeptide. To illustrate, the polypeptides may be provided in a kit, such as a kit in which the polypeptides are contained in separate containers, and optionally further comprising  
20       an instruction sheet providing guidance on performance of the assay. The contents can then be employed for measuring the p110δ:LASP-1 interaction in a modulator identification assay. For HTS assays, the invention provides multi-well (e.g., 96- or 384-well) plates in which at least one well contains a p110δ polypeptide and a LASP-1 polypeptide in amounts suitable for screening small quantities of a test compound.  
25       Alternatively, for cell-based assays, the invention provides containers (e.g., multi-well plates) containing host cells recombinantly modified to express, preferably cells modified to coexpress, a p110δ polypeptide and a LASP-1 polypeptide.

Therapeutic Uses of Inhibitors of p110δ:LASP-1 Binding

As described herein, the invention provides a method for identifying compounds capable of selectively modulating p110δ:LASP-1 binding, or modulating PI3Kδ activity mediated thereby. Thus, the invention may provide the basis for development of methods of treatment of PI3Kδ-mediated disease states. Such treatment methods can include administering an modulator, most probably an inhibitor, of p110δ:LASP-1 binding in an amount effective for this purpose. These methods may be employed in treating humans or animals that are or may be subject to any disease state whose symptoms or pathology is characterized by PI3Kδ activity as it may be mediated by p110δ:LASP-1 binding. Among other things, as PI3Kδ expression is predominantly limited to leukocytes, the invention enables methods of inhibiting PI3Kδ function in these cells. Thus, disease states in which leukocyte function is excessive or undesirable may be amenable to treatment using p110δ:LASP-1 binding modulators as described hereinbelow in greater detail.

In one aspect, the invention provides methods of treating disease states characterized by functions of neutrophils. It has been observed that inhibition of PI3Kδ activity inhibits certain functions of neutrophils such as stimulated superoxide release, stimulated exocytosis, and chemotactic migration. Superoxide is released by neutrophils in response to any of a variety of stimuli including signals of infection, as a mechanism of cell killing. For example, superoxide release is known to be induced by tumor necrosis factor alpha (TNFα), which is released by macrophages, mast cells, and lymphocytes upon contact with bacterial cell wall components such as lipopolysaccharide (LPS). TNFα is an extraordinarily potent and promiscuous activator of inflammatory processes, being involved in activation of neutrophils and various other cell types, induction of leukocyte/endothelial cell adhesion, pyrexia, enhanced MHC class I production, and stimulation of angiogenesis. Alternatively, superoxide release may be stimulated by formyl-Met-Leu-Phe (fMLP) or other peptides blocked at the N-terminus by formylated methionine. Such peptides are not normally found in eukaryotes, but are fundamentally characteristic of bacteria, and signal the presence of bacteria to the immune system. Leukocytes expressing the fMLP receptor, e.g., neutrophils and macrophages, are stimulated to migrate up

gradients of these peptides (i.e., chemotaxis) toward loci of infection. In general, these functions may be collectively termed "inflammatory functions," as they are typically related to neutrophil response to inflammation. Such functions may further include, without limitation, stimulated degranulation, adhesion to vascular endothelium (e.g., tethering/rolling of neutrophils, triggering of neutrophil activity, and/or latching of neutrophils to endothelium), transmural diapedesis or emigration through the endothelium to peripheral tissues. The inflammatory functions of neutrophils may be distinguished from the bacterial killing functions exhibited by these cells, e.g., phagocytosis and killing of bacteria.

Without intending to be bound by any one theory, it is believed that, because inflammation involves processes are typically mediated by leukocyte (e.g., neutrophil, lymphocyte, etc.) activation and chemotactic transmigration, and because PI3K $\delta$  may mediate such phenomena, antagonists of PI3K $\delta$  activity mediated by p110 $\delta$ :LASP-1 binding may be used to suppress injury associated with inflammation. Accordingly, the invention further includes methods of treating disease states in which one or more of the inflammatory functions of neutrophils are abnormal or undesirable. Neutrophil functions suitable for inhibition according to the method include any function that is characterized by PI3K $\delta$  activity mediated by p110 $\delta$ :LASP-1 binding.

It has further been observed that PI3K $\delta$  plays a role in the stimulated proliferation of lymphocytes, including B cells and T cells. Moreover, PI3K $\delta$  appears to play a role in stimulated secretion of antibodies by B cells. Thus, the invention includes methods for inhibiting lymphocyte proliferation, and methods for inhibiting antibody production by B lymphocytes through the use of modulators of p110 $\delta$ :LASP-1 binding. Other methods enabled by the invention include methods of treating disease states in which one or more of these lymphocyte functions are abnormal or undesirable.

In another aspect, the invention includes a method for suppressing a function of basophils and/or mast cells, and thereby enabling treatment of disease states characterized by excessive or undesirable basophil and/or mast cell activity.

According to the method, a compound of the invention may be used that selectively inhibits p110 $\delta$ :LASP-1 binding and associated activity of phosphatidylinositol 3-kinase delta (PI3K $\delta$ ) in basophils and/or mast cells. Preferably, the method employs

a p110 $\delta$ :LASP-1 inhibitor in an amount sufficient to inhibit stimulated histamine release by the basophils and/or mast cells. Accordingly, the use of such compounds and other PI3K $\delta$  selective inhibitors may be of value in treating diseases characterized by histamine release, i.e., allergic disorders, including disorders such as chronic obstructive pulmonary disease (COPD), asthma, ARDS, emphysema, and related disorders.

In another aspect, selective inhibitors of PI3K $\delta$  activity mediated by p110 $\delta$ :LASP-1 binding may be employed in methods of treating diseases of bone, especially diseases in which osteoclast function is abnormal or undesirable. Accordingly, the use of such compounds may be of value in treating osteoporosis, Paget's disease, and related bone resorption disorders.

In a further aspect, the invention includes methods of using modulators of p110 $\delta$ :LASP-1 binding to inhibit the growth or proliferation of cancer cells of hematopoietic origin, preferably cancer cells of lymphoid origin, and more preferably cancer cells related to or derived from B lymphocytes or B lymphocyte progenitors. Cancers potentially amenable to treatment using the method of the invention include, without limitation, lymphomas, i.e., malignant neoplasms of lymphoid and reticuloendothelial tissues, such as Burkitt's lymphoma, Hodgkins' lymphoma, non-Hodgkins lymphomas, lymphocytic lymphomas and the like; multiple myelomas; as well as leukemias such as lymphocytic leukemias, chronic myeloid (myelogenous) leukemias, and the like. In a preferred embodiment, p110 $\delta$ :LASP-1 modulatory compounds may be used to inhibit or control the growth or proliferation of chronic myeloid (myelogenous) leukemia cells.

"Treating" as used herein refers to preventing a disease state from occurring in an animal that may be predisposed to the disease state, but has not yet been diagnosed as having it; inhibiting the disease state, i.e., arresting its development; relieving the disease state, i.e., causing its regression; or ameliorating the disease state, i.e., reducing the severity of symptoms associated with the disease state.

"Disease state" as used herein is intended to encompass pathological disorders, diseases, conditions, syndromes, and the like, without limitation.

"Inflammatory disease" as used herein can refer to any disease state in which an excessive or unregulated inflammatory response leads to excessive inflammatory

symptoms, host tissue damage, or loss of tissue function. "Inflammatory disease" also refers to a pathological state mediated by influx of leukocytes and/or neutrophil chemotaxis.

5 "Inflammation" as used herein refers to a localized, protective response elicited by injury or destruction of tissues, which serves to destroy, dilute, or wall off (sequester) both the injurious agent and the injured tissue. Inflammation is notably associated with influx of leukocytes and/or neutrophil chemotaxis. Inflammation may result from infection with pathogenic organisms and viruses and from noninfectious means such as trauma or reperfusion following myocardial infarction or stroke,  
10 immune response to foreign antigen, and autoimmune responses. Accordingly, inflammatory diseases amenable to the invention encompass disease states associated with reactions of the specific defense system as well as with reactions of the non-specific defense system.

15 "Specific defense system" as used herein refers to the component of the immune system that reacts to the presence of specific antigens. Examples of inflammation resulting from a response of the specific defense system include the classical response to foreign antigens, autoimmune diseases, and delayed type hypersensitivity response mediated by T-cells. Chronic inflammatory diseases, the rejection of solid transplanted tissue and organs, e.g., kidney and bone marrow  
20 transplants, and graft versus host disease (GVHD), are further examples of inflammatory reactions of the specific defense system.

25 "Non-specific defense system" as used herein refers to the cells systems involved in inflammatory processes that are mediated by leukocytes that are incapable of immunological memory (e.g., granulocytes, and macrophages). Examples of inflammation that result, at least in part, from a reaction of the non-specific defense system include inflammation associated with conditions such as adult (acute) respiratory distress syndrome (ARDS) or multiple organ injury syndromes; reperfusion injury; acute glomerulonephritis; reactive arthritis; dermatoses with acute  
30 inflammatory components; acute purulent meningitis or other central nervous system inflammatory diseases such as stroke; thermal injury; inflammatory bowel disease; granulocyte transfusion associated syndromes; and cytokine-induced toxicity.



“Autoimmune disease” as used herein refers to any group of disease states in which tissue injury is associated with humoral or cell-mediated responses to the body’s own constituents. “Allergic disease” as used herein refers to any disease state in which symptoms, tissue damage, or loss of tissue function result from allergy.

5 “Arthritic disease” as used herein refers to any disease state that is characterized by inflammatory lesions of the joints attributable to a variety of etiologies. “Dermatitis” as used herein refers to any of a large family of diseases of the skin that are characterized by inflammation of the skin attributable to a variety of etiologies.

10 “Transplant rejection” as used herein refers to any immune reaction directed against grafted tissue, such as organs or cells (e.g., bone marrow), characterized by a loss of function of the grafted and surrounding tissues, pain, swelling, leukocytosis, and thrombocytopenia.

The therapeutic methods of the present invention include methods for the treatment of disease states associated with inflammatory cell activation.

15 “Inflammatory cell activation” refers to the induction by a stimulus (including, but not limited to, cytokines, antigens or autoantibodies) of a proliferative cellular response, the production of soluble mediators (including but not limited to cytokines, oxygen radicals, enzymes, prostanoids, or vasoactive amines), or cell surface expression of new or increased numbers of mediators (including, but not limited to, major histocompatibility antigens or cell adhesion molecules) in inflammatory cells  
20 (including but not limited to monocytes, macrophages, T lymphocytes, B lymphocytes, granulocytes (i.e., polymorphonuclear leukocytes such as neutrophils, basophils, and eosinophils), mast cells, dendritic cells, Langerhans cells, and endothelial cells). It will be appreciated by persons skilled in the art that the  
25 activation of one or a combination of these phenotypes in these cells can contribute to the initiation, perpetuation, or exacerbation of an inflammatory disease.

The present invention enables methods of treating such diseases as arthritic diseases, such as rheumatoid arthritis, monoarticular arthritis, osteoarthritis, gouty arthritis, spondylitis; Behcet disease; sepsis, septic shock, endotoxic shock, gram  
30 negative sepsis, gram positive sepsis, and toxic shock syndrome; multiple organ injury syndrome secondary to septicemia, trauma, or hemorrhage; ophthalmic disorders such as allergic conjunctivitis, vernal conjunctivitis, uveitis, and thyroid-

associated ophthalmopathy; eosinophilic granuloma; pulmonary or respiratory disorders such as asthma, chronic bronchitis, allergic rhinitis, ARDS, chronic pulmonary inflammatory disease (e.g., chronic obstructive pulmonary disease), silicosis, pulmonary sarcoidosis, pleurisy, alveolitis, vasculitis, emphysema, pneumonia, bronchiectasis, and pulmonary oxygen toxicity; reperfusion injury of the myocardium, brain, or extremities; fibrosis such as cystic fibrosis; keloid formation or scar tissue formation; atherosclerosis; autoimmune diseases such as systemic lupus erythematosus (SLE), autoimmune thyroiditis, multiple sclerosis, some forms of diabetes, and Reynaud's syndrome; and transplant rejection disorders such as GVHD and allograft rejection; chronic glomerulonephritis; inflammatory bowel diseases such as chronic inflammatory bowel disease (CIBD), Crohn's disease, ulcerative colitis, and necrotizing enterocolitis; inflammatory dermatoses such as contact dermatitis, atopic dermatitis, psoriasis, or urticaria; fever and myalgias due to infection; central or peripheral nervous system inflammatory disorders such as meningitis, encephalitis, and brain or spinal cord injury due to minor trauma; Sjögren's syndrome; diseases involving leukocyte diapedesis; alcoholic hepatitis; bacterial pneumonia; antigen-antibody complex mediated diseases; hypovolemic shock; Type I diabetes mellitus; acute and delayed hypersensitivity; disease states due to leukocyte dyscrasia and metastasis; thermal injury; granulocyte transfusion-associated syndromes; and cytokine-induced toxicity.

The method can have utility in treating subjects that are or may be subject to reperfusion injury, i.e., injury resulting from situations in which a tissue or organ experiences a period of ischemia followed by reperfusion. The term "ischemia" refers to localized tissue anemia due to obstruction of the inflow of arterial blood. Transient ischemia followed by reperfusion characteristically results in neutrophil activation and transmigration through the endothelium of the blood vessels in the affected area. Accumulation of activated neutrophils in turn results in generation of reactive oxygen metabolites, which damage components of the involved tissue or organ. This phenomenon of "reperfusion injury" is commonly associated with conditions such as vascular stroke (including global and focal ischemia), hemorrhagic shock, myocardial ischemia or infarction, organ transplantation, and cerebral vasospasm. To illustrate, reperfusion injury occurs at the termination of cardiac

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bypass procedures or during cardiac arrest when the heart, once prevented from receiving blood, begins to reperfuse. It is expected that inhibition of PI3K $\delta$  activity will result in reduced amounts of reperfusion injury in such situations.

5 With respect to the nervous system, global ischemia occurs when blood flow to the entire brain ceases for a period. Global ischemia may result from cardiac arrest. Focal ischemia occurs when a portion of the brain is deprived of its normal blood supply. Focal ischemia may result from thromboembolic occlusion of a cerebral vessel, traumatic head injury, edema, or brain tumor. Even if transient, both  
10 global and focal ischemia can cause widespread neuronal damage. Although nerve tissue damage occurs over hours or even days following the onset of ischemia, some permanent nerve tissue damage may develop in the initial minutes following the cessation of blood flow to the brain.

Ischemia can also occur in the heart in myocardial infarction and other cardiovascular disorders in which the coronary arteries have been obstructed as a  
15 result of atherosclerosis, thrombus, or spasm. Accordingly, the invention is believed to be useful for treating cardiac tissue damage, particularly damage resulting from cardiac ischemia or caused by reperfusion injury in mammals.

The methods of the invention embrace various modes of treating an animal, preferably a mammal, more preferably a primate, and still more preferably a human.  
20 Among the mammals that may be treated are, for example, companion animals (pets) including dogs and cats; farm animals including cattle, horses, sheep, pigs, and goats; laboratory animals including rats, mice, rabbits, guinea pigs, and non-human primates. Non-mammalian animals include, for example, birds, fish, reptiles, and amphibians.

25

Methods of Administration of Modulators of p110 $\delta$ :LASP-1 Interaction

Pharmaceutical compositions comprising a modulator of p110 $\delta$ :LASP-1 binding activity may be administered to the subject by any conventional method, including parenteral and enteral techniques. Parenteral administration modalities include those in which the composition is administered by a route other than through the gastrointestinal tract, for example, intravenous, intraarterial, intraperitoneal, intramedullary, intramuscular, intraarticular, intrathecal, and intraventricular injections. Enteral administration modalities include, for example, oral (including buccal and sublingual) and rectal administration. Transepithelial administration modalities include, for example, transmucosal administration and transdermal administration. Transmucosal administration includes, for example, enteral administration as well as nasal, inhalation, and deep lung administration; vaginal administration; and rectal administration. Transdermal administration includes passive or active transdermal or transcutaneous modalities, including, for example, patches and iontophoresis devices, as well as topical application of pastes, salves, or ointments. Parenteral administration can also be accomplished using a high-pressure technique, e.g., POWDERJECT®. Surgical techniques include implantation of depot (reservoir) compositions, osmotic pumps, and the like. A preferred route of administration for treatment of inflammation can be local or topical delivery for localized disorders such as arthritis, or systemic delivery for distributed disorders, e.g., intravenous delivery for reperfusion injury or for systemic conditions such as septicemia. For other diseases, including those involving the respiratory tract, e.g., chronic obstructive pulmonary disease, asthma, emphysema, etc., administration may be accomplished by inhalation or deep lung administration of sprays, aerosols, powders, and the like.

For the treatment of neoplastic diseases, especially leukemias and other distributed cancers, parenteral administration is typically preferred. Formulations of the compounds to optimize them for biodistribution following parenteral administration would be desirable. The PI3K $\delta$  inhibitor compounds may be administered before, during, or after administration of chemotherapy, radiotherapy, and/or surgery.

Moreover, the therapeutic index of the p110 $\delta$ :LASP-1 modulator compounds may be enhanced by modifying or derivatizing the compounds for targeted delivery to cancer cells expressing a marker that identifies the cells as such. For example, the compounds may be linked to an antibody that recognizes a marker that is selective or specific for cancer cells, so that the compounds are brought into the vicinity of the cells to exert their effects locally, as previously described [see for example, Pietersz et al., *Immunol Rev* 129:57 (1992); Trail et al., *Science* 261:212 (1993); and Rowlinson-Busza et al., *Curr Opin Oncol* 4:1142 (1992)]. Tumor-directed delivery of these compounds would enhance the therapeutic benefit by, inter alia, minimizing potential non-specific toxicities that can result from radiation treatment or chemotherapy. In another aspect, p110 $\delta$ :LASP-1 inhibitor compounds and radioisotopes or chemotherapeutic agents may be conjugated to the same anti-tumor antibody.

For the treatment of bone resorption disorders or osteoclast-mediated disorders, the p110 $\delta$ :LASP-1 modulators can be delivered by any suitable method. Focal administration may be desirable, such as by intraarticular injection. In some cases, it may be desirable to couple the compounds to a moiety that can target the compounds to bone. For example, a p110 $\delta$ :LASP-1 modulator may be coupled to compounds with high affinity for hydroxyapatite, which is a major constituent of bone. This may be accomplished, for example, by adapting a tetracycline-coupling method developed for targeted delivery of estrogen to bone [Orme et al., *Bioorg Med Chem Lett* 4(11):1375-80 (1994)].

To be effective therapeutically in modulating central nervous system targets, the agents used in the methods of the invention should readily penetrate the blood brain barrier when peripherally administered. Compounds that cannot penetrate the blood brain barrier, however, can still be effectively administered by an intravenous route.

As noted above, the characteristics of the agent itself and the formulation of the agent can influence the physical state, stability, rate of *in vivo* release, and rate of *in vivo* clearance of the administered agent. Such pharmacokinetic and pharmacodynamic information can be collected through pre-clinical *in vitro* and *in vivo* studies, later confirmed in humans during the course of clinical trials. Thus, for

any compound used in the method of the invention, a therapeutically effective dose can be estimated initially from biochemical and/or cell-based assays. Then, dosage can be formulated in animal models to achieve a desirable circulating concentration range that modulates p110 $\delta$ :LASP-1 binding. As human studies are conducted,  
5 further information will emerge regarding the appropriate dosage levels and duration of treatment for various diseases and conditions.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD<sub>50</sub> (the dose lethal to 50% of the population) and the ED<sub>50</sub> (the  
10 dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the "therapeutic index," which is typically expressed as the ratio LD<sub>50</sub>/ED<sub>50</sub>. Compounds that exhibit large therapeutic indices are preferred. The data obtained from such cell culture assays and additional animal studies can be used in formulating a range of dosage for human use. The dosage of  
15 such compounds lies preferably within a range of circulating concentrations that include the ED<sub>50</sub> with little or no toxicity.

For the method of the invention, any effective administration regimen regulating the timing and sequence of doses may be used. Doses of the agent preferably include pharmaceutical dosage units comprising an effective amount of the  
20 agent. As used herein, "effective amount" refers to an amount of a p110 $\delta$ :LASP-1 modulator compound sufficient to modulate PI3K $\delta$  activity and/or derive a measurable change in a physiological parameter of the subject through administration of one or more of the pharmaceutical dosage units.

Exemplary dosage levels for a human subject are of the order of from about  
25 0.001 milligram of active agent per kilogram body weight (mg/kg) to about 100 mg/kg. Typically, dosage units of the active agent comprise from about 0.01 mg to about 10,000 mg, preferably from about 0.1 mg to about 1,000 mg, depending upon the indication, route of administration, etc. Depending on the route of administration, a suitable dose may be calculated according to body weight, body surface area, or  
30 organ size. The final dosage regimen will be determined by the attending physician in view of good medical practice, considering various factors that modify the action of drugs, e.g., the agent's specific activity, the severity of the disease state, the

responsiveness of the patient, the age, condition, body weight, sex, and diet of the patient, the severity of any infection, etc. Additional factors that may be taken into account include time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Further refinement of the dosage  
5 appropriate for treatment involving any of the formulations mentioned herein is done routinely by the skilled practitioner without undue experimentation, especially in light of the dosage information and assays disclosed, as well as the pharmacokinetic data observed in human clinical trials. Appropriate dosages may be ascertained through use of established assays for determining concentration of the agent in a body fluid or  
10 other sample together with dose response data.

The frequency of dosing will depend on the pharmacokinetic parameters of the agent and the route of administration. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Accordingly, the pharmaceutical compositions can be administered in a single dose,  
15 multiple discrete doses, continuous infusion, sustained release depots, or combinations thereof, as required to maintain desired minimum level of the agent. Short-acting pharmaceutical compositions (i.e., short half-life) can be administered once a day or more than once a day (e.g., two, three, or four times a day). Long acting pharmaceutical compositions might be administered every 3 to 4 days, every  
20 week, or once every two weeks. Pumps, such as subcutaneous, intraperitoneal, or subdural pumps, may be preferred for continuous infusion.

The following Examples are provided to further aid in understanding the invention, and presuppose an understanding of conventional methods well-known to those persons having ordinary skill in the art to which the examples pertain, e.g., the  
25 construction of vectors and plasmids, the insertion of genes encoding polypeptides into such vectors and plasmids, or the introduction of vectors and plasmids into host cells. Such methods are described in detail in numerous publications including, for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press (1989), Ausubel et al. (Eds.), *Current Protocols in*  
30 *Molecular Biology*, John Wiley & Sons, Inc. (1994); and Ausubel et al. (Eds.), *Short Protocols in Molecular Biology*, 4<sup>th</sup> ed., John Wiley & Sons, Inc. (1999). The particular materials and conditions described hereunder are intended to exemplify

particular aspects of the invention and should not be construed to limit the reasonable scope thereof.

### EXAMPLE 1. Identification and Verification of a p110 $\delta$ -Interacting Partner

The yeast di-hybrid technique was used in an effort to identify proteins that interact with the 110 kDa subunit of PI3 kinase delta (p110 $\delta$ ). The di-hybrid method is described by Fields et al. [*Nature* 340(6230):245-6 (1989)]. [See also, e.g., U.S. Patent No. 5,959,079; Hollenberg et al., *Mol Cell Biol* 15:3813-22 (1995)].

#### Construction of Bait Plasmid and Yeast Strains

As shown in Table 1, a comparison of the amino acid sequence of human p110 $\delta$  to those of the other three p110 subunit types reveals that the amino acid sequence of the Ras-binding domain is a less conserved region than the catalytic domain.

Table 1

PI3K Isoform	Overall	Identity	
		Catalytic	Ras-Binding
p110 $\alpha$ (SEQ ID NO:6)	41	49	26
p110 $\beta$ (SEQ ID NO:8)	58	72	35.5
p110 $\gamma$ (SEQ ID NO:10)	35	45	20

To increase the probability of identifying proteins that specifically interact with p110 $\delta$ , we took advantage of the low level of homology in the Ras-binding domains. The Ras-binding region of p110 $\delta$  (aa 134 to 350 of SEQ ID NO:2) was amplified by PCR using 100 ng of template p110 $\delta$  DNA (SEQ ID NO:1) and 500 ng each of the following primers.



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p110delta 5': 5'-GATCGAATTCCCAGAAGTGAACGACTTTCGC-3'

(SEQ ID NO:11)

p110delta 3': 5'-GATCGTCGACGCCGTGGAAAAGCCCGGCCTG-3'

(SEQ ID NO:12)

5

The amplification reaction was performed using Taq polymerase and buffer provided by the supplier (Perkin Elmer, Foster City CA) according to the following temperature cycling protocol: 94°C for 5 min; followed by 25 cycles of 94°C for 30 sec, 68°C for 3 min, and 72° for 7 min. The amplified product was then digested with *Eco*R1 and *Sal*I and subcloned in the *Eco*R1-*Sal*I sites of the yeast di-hybrid vector pBTM116 such that the p110δ sequence was in frame with the LexA sequence.

10

We also cloned the corresponding region of p110β (aa 143 to 360) in frame with the LexA sequence for use as a specificity control. The following oligonucleotides were used to amplify the Ras-binding domain of p110β sequence (SEQ ID NO:8).

15

p110beta 5 : 5 -GATCGAATTCCCTGAAGTAAATGAATTTCTGA-3

(SEQ ID NO:13)

p110beta 3 : 5 -GATCGTCGACACCATGAAAAAGACCAGCCCT-3

20

(SEQ ID NO:14)

The pBTM116 plasmid DNAs containing the p110δ or p110β sequences were then introduced into yeast cells by the lithium acetate treatment, generally in accordance with the method of Ito et al. [*J Bacteriol* 153:163-8 (1983)].

25

#### Screening for p110δ-Interacting Clones

One yeast colony containing the p110δ Ras-binding sequence was grown and the cells were transformed with a mouse embryonic cDNA library in the yeast vector pVP16. In this vector, the mouse cDNAs are cloned in frame with the VP16 sequence so that the resulting fusion protein can recognize RNA polymerase using the VP16 sequence to help initiate transcription. The cells were plated on appropriate selection medium lacking leucine, tryptophan and histidine. The ability of the yeast transformants to survive and propagate on this medium suggests an interaction

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between p110 $\delta$  and the protein coded by the mouse cDNA sequence. As a further test of protein:protein interaction, replicas of the yeast colonies on the selection plates were made on membranes and assayed for  $\beta$ -galactosidase activity by a standard method [Breedon et al. *Cold Spring Harbor Symp Quant Biol* 50:643-50 (1985)].  
5 Several hundred blue colonies were observed. The colonies that did not turn blue were considered false positive and discarded.

We selected 40 of the colonies that turned blue most rapidly and isolated plasmid DNA from each of them. *E. coli* cells that require leucine for growth were transformed with each of these 40 plasmid DNAs and plated on medium lacking  
10 leucine so that the cells carrying VP16 based plasmids would be selected. Plasmid DNAs were isolated from the *E. coli* transformants and re-introduced into the yeast cells carrying pBTM116-p110 $\delta$  Ras-binding domain. The resulting yeast transformants were again tested for the interaction with p110 $\delta$  by growth on medium lacking leucine, tryptophan, and histidine, and also assayed for  $\beta$ -galactosidase  
15 activity. Only those colonies that reproducibly tested positive for the interaction were selected for further characterization.

### Characterization of p110 $\delta$ -Interacting Clones

#### Sequence Analysis

We determined the DNA sequences of 39 clones that were reproducibly positive for interaction with the p110 $\delta$  region of interest (aa 134 to 350 of SEQ ID NO:2). All of the DNA sequences were used to search the GenBank® database for any related proteins. Our searches revealed that the DNA sequence of Clone 32 (SEQ ID NO:15) is highly homologous with portions of the human and mouse LASP-1  
25 DNAs (GenBank® Accession Nos. NM\_006148 (human; SEQ ID NO:3) and NM\_010688 (mouse; SEQ ID NO:17). The LASP-1 proteins of rabbit, human and mouse contain 2 domains: one LIM motif and one domain of Src homology region 3 (SH3), hence its name "LIM and SH3 Protein" [Tomasetto et al., *FEBS Lett* 373:245-9 (1995); Chew et al., *Amer J Physiol* 275 (Cell Physiol 44):C56-C57 (1998)]. The  
30 amino acid sequence of the SH3 domain of Clone 32 (aa 4-60 of SEQ ID NO:16) was identical to the SH3 domain of both the human and mouse LASP-1 proteins (aa 205-261 of SEQ ID NO:4 and aa 207-263 of SEQ ID NO:18, respectively), which are

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identical to one another. Moreover, the amino acid sequences of the Ras-binding domains of mouse and human p110 are 95% conserved. In particular, the amino acid sequences of the proline-rich segments within the Ras-binding domains of both the human and mouse p110 $\delta$  protein are identical. Since the mouse and human LASP-1 SH3 domain amino acid sequences are identical, and since the mouse and human p110 $\delta$  proline-rich segments are identical, we surmise that human LASP-1 is capable of binding to the human p110 $\delta$  proline-rich segment as does clone 32 which is derived from mouse LASP-1.

The DNA sequence of the Clone 32 is given below (note that the *NotI* sites (underlined) at the 5' and 3' ends are from the vector).

GCGGCCGCTCAAACGGTACCGTGCA GTGTATGACTACAGCGCTGCCGACGAGGACGA  
GGTCTCCTTCCAGGATGGGGACACCATCGTCAATGTGCAGCAGATCGATGACGGCTG  
GATGTACGGGACCGTAGAGCGCACCGGTGACACGGGGATGCTGCCAGCCAACTACGT  
GGAGGCCATCTGAACCCTGTGCCGCCCGCCCTGTCTTCAATGCATTCCATGGCATC  
ACATCTGTCTTGGGGCCTGACCCGTCCACCCTACAGTGTCTCTGTCTTTAAGATCT  
TCAACTGCTTCTTTATCCCCGCCCTCCAGCTTATTTTACCATCCCAAGCCTTGTTCTG  
TGCCCCCTGAGGCGGCCGCT

(SEQ ID NO:15)

Since the mouse cDNAs are fused to the coding sequence of VP16, we deduced that the polypeptide sequence encoded by Clone 32 (below) was in the same reading frame as VP16. As noted above, amino acids 4-60 of SEQ ID NO:16 correspond to the amino terminal region of LASP-1.

RPLKRYRAVYDYSAADEDEVSFQDGD TIVNVQQIDDGWMYGTVERTGDTGMLPANYV  
EAI

(SEQ ID NO:16)

Test for Specificity of Interaction of p110 $\delta$  with Clone 32

To check for the specificity of interaction of Clone 32 with the PI3 kinases, we introduced the Clone 32 plasmid DNA into the yeast cells carrying the p110 $\beta$  Ras-binding domain, since the p110 $\beta$  sequence is closest to the p110 $\delta$  sequence. After selection on appropriate growth medium, the yeast transformants were assayed for  $\beta$ -galactosidase activity. The cells carrying the p110 $\beta$  sequence did not show any  $\beta$ -galactosidase activity whereas the p110 $\delta$ -carrying cells again showed positive activity, suggesting an absence of interaction between Clone 32 and p110 $\beta$  Ras-binding domain (Table 2).

Clone 32 Interacts with the Proline (Pro) Rich Region of p110 $\delta$ 

As determined by sequence analysis, Clone 32 comprises an SH3 domain. Since SH3 domains are known to bind proline rich sequences, we tested whether Clone 32 binds to the proline rich sequence of p110 $\delta$ . We created 3 individual clones, each carrying one of the following mutations: 1) P304/A, 2) P307/A, and 3) deletion of 51 amino acids (aa 300-350 of SEQ ID NO:2) into our original pBTM116-p110 $\delta$  Ras-binding domain construct using standard recombinant DNA techniques. Sequences of the proline-rich segments of p110 $\beta$  and p110 $\delta$  are given below. In the p110 $\delta$  sequence, the proline residues mutated to provide the P304/A and P307/A clones are identified.

p110 $\delta$ : EQSNPAPQVQKPRAK----PPPIPAKKPSSV

(aa 288-314 of SEQ ID NO:2)

p110 $\beta$ : EQEMIAIEAAINRNSNLPLPLPPKKTRIIS

(aa 294-324 of SEQ ID NO:8)

The three mutant plasmids were introduced into yeast cells and tested for interaction with Clone 32 by growth on selective medium and  $\beta$ -galactosidase activity. Growth of the cells containing Clone 32 or mutant plasmids were poor. Moreover, as shown in Table 2, change of any of the proline residues to alanine

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resulted in a reduction of  $\beta$ -galactosidase activity. Deletion of the proline rich segment eliminated  $\beta$ -galactosidase activity. These results suggest that the Clone 32 (which encodes an SH3 domain) product interacts with p110 $\delta$  (aa 134-350 of SEQ ID NO:2) through the proline rich segment of p110 $\delta$ .

Table 2

Bait	$\beta$ -Galactosidase Activity
p110 $\beta$	-
p110 $\delta$	++
p110 $\delta$ (P304/A)	-
p110 $\delta$ (P307/A)	+/-
p110 $\delta$ (Pro Rich Segment Deletion)	-

#### Further Verification of Interaction of p110 $\delta$ Ras-Binding Domain with Clone 32

Since the yeast di-hybrid technique can produce protein:protein interactions that are not always reproducible in mammalian cells, we tested whether Clone 32 can interact with p110 $\delta$  within a mammalian cell environment. We generated a fusion of GFP (Green Florescent Protein) with Clone 32 in the vector pcDNA3.1/NT-GFP-TOPO (Invitrogen, San Diego, CA) according to the procedure recommended by the supplier. The resulting fusion plasmid, designated "GFP-32," was co-transfected in triplicate with a FLAG-p110 $\delta$ -containing plasmid [Chantry et al., *J Biol Chem* 272:19236-41 (1997)] into the human embryonic kidney cell line HEK293. (The FLAG® N-terminal peptide identification system is described e.g., in US Patent Nos. 4,851,341 and 5,011,912.) Forty-eight hours after transfection the cells were lysed and immunoprecipitated using anti-FLAG® antibody M2-coated agarose beads. The bound proteins were separated by SDS-PAGE, blotted on nylon membrane, and probed with a rabbit anti-GFP antibody (Clontech, Palo Alto CA) and rabbit anti-p110 $\delta$  antibodies (prepared using the method described in US Patent No. 5,882,910). A horseradish peroxidase (HRP)-linked goat anti-rabbit antibody (Clontech) was used according to the manufacturer's protocol to develop the blot.

Examination of the developed blot showed a band at about 33 kDa in the lanes containing lysates of cells transfected with the plasmid pcDNA3.1/NT-GFP, indicating expression of the GFP protein only. In the lanes in which lysates from FLAG-p110 $\delta$  and pcDNA3.1/NT-GFP-clone32 co-transfected cells were applied, both the FLAG-p110 $\delta$  band (at about 110 kDa) and the GFP-clone32 band (at about 40 kDa) were observed, indicating expression of both proteins.

We also analyzed the supernatant and pellet of anti-FLAG® immunoprecipitated samples for the presence of FLAG-p110 $\delta$  and GFP-clone32. As expected, the supernatants contained very little FLAG-p110 $\delta$  signal, indicating successful precipitation of FLAG-p110 $\delta$ . Complementing the absence of FLAG-p110 $\delta$  in the supernatant, there was a large increase in the FLAG-p110 $\delta$  signal in the pellets. In addition to the FLAG-p110 $\delta$  signal, another band was observed in the pellet. This band comigrated with the GFP-clone32 band. These results indicate that Clone 32 can associate with p110 $\delta$  within the mammalian cellular environment.

LASP-1 has been shown to be phosphorylated in response to extracellular signals such as forskolin, and the phosphorylation is correlated to the secretory response of the cells [Chew et al., *Am J Physiol* 275 (*Cell Physiol* 44:C56-C57) (1998)]. In addition, LASP-1 has been shown to be tyrosine phosphorylated by c-Src and binds to actin [Schreiber et al., *Mol Med* 4:675-687 (1998)]. Since p110 $\delta$  is involved in cellular signal transduction pathways, we surmise that the LASP-1: p110 $\delta$  interaction may facilitate secretion and/or cytoskeletal reorganization. Disruption of the interaction between p110 $\delta$  proline rich sequence and SH3 domains may prevent p110 $\delta$  activation in response to specific stimulus in a given cell type and thereby prevent unwanted signaling through the p110 $\delta$  pathway. Disease states mediated by excessive or undesirable p110 $\delta$  activity, therefore, may be expected to be susceptible to treatment using modulator compounds that disrupt binding or interaction of p110 $\delta$  with LASP-1.

#### EXAMPLE 2. Functional Significance of the Proline-rich Sequence in p110 $\delta$

SH3 domains are approximately sixty amino acid residues long and are found in many signaling proteins, enzymes, and cytoskeletal proteins. Despite their amino

acid sequence diversity, all SH3 domains bind to a short stretch of polyproline sequences of 8 to 10 amino acid residues. Even though several hundred distinct SH domains are known in human, they seem to bind polyproline sequences with a remarkable degree of specificity. The interaction between a polyproline sequence and a SH3 domain may be intramolecular or intermolecular. In the former case, the polyproline sequence and the SH3 domain of the same polypeptide chain bind to each other. In case of intermolecular, interactions the polyproline sequence and the SH3 domain from different proteins interact.

Polyproline sequences serve as ligands for many protein domains such as the EVH1 (Enabled, VASP Homology 1), WW and SH3 (Src Homology 3) for example. These protein domains and their ligand polyproline sequences have been observed in many species suggesting their evolutionary conserved role in cellular functions. Among the different domains that bind polyproline sequences, the SH3 domain has been studied most of all.

SH3 binding has been shown to activate signaling enzymes as well as in the formation of active signaling complexes [Pleiman et al., *Science* 263:1609-1612 (1994); Pawson, *Nature* 373:573-580 (1995)]. The present study shows that the proline-rich segment of p110 $\delta$  can function as an SH3 binding sequence. Hence, it is possible that binding of SH3 domain-containing proteins leads to the activation of the kinase activity of p110 $\delta$ . For example, Src family kinases and adapter proteins (e.g., vav, cbl) having one or more SH3 domains may bind to p110 $\delta$  through the proline-rich domain.

Due to the important roles of polyproline sequences in the regulation of cellular activation, small molecule inhibitors are being developed to prevent the interaction between polyproline sequences and their cognate binding partners. Proline is the only amino acid where the amido N is substituted. Based on this unique feature of proline, Nguyen et al., *Science*, 282: 2088-2092 (1998) designed a set of N-substituted analogs that can inhibit SH3-polyproline interaction.

#### Indications for p110 $\delta$ Activity Requirement in Neutrophils

Treatment of neutrophils with a p110 $\delta$ -specific inhibitor (such as disclosed in U.S.S.N. 09/841,341 filed April 24, 2001, the disclosure of which is hereby

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incorporated by reference) results in the inhibition of neutrophil functions such as superoxide production. NADPH oxidase, the enzyme responsible for superoxide production, is a multicomponent enzyme, and p47-phox is an essential constituent of the complex. P47-phox is described in Volpp et.al., *PNAS*, 86: 7195-7199 (1989).

5 The p47-phox protein contains two SH3 domains. Human mutations of p47-phox have been discovered that result in defects in superoxide production by neutrophils. Most of these p47-phox mutations cause premature truncation and as a result the SH3 domains are not synthesized. These observations establish that in addition to p110δ, p47-phox is also necessary for superoxide production. Since, both p110δ and p47-phox are required, it is contemplated that the polyproline sequence of p110δ functions in superoxide synthesis through an interaction with SH3 domain of p47-phox.

#### Indication for p110δ Activity Requirement in B cells

15 In addition to inhibiting neutrophil function, p110δ-specific inhibitor blocks B cell proliferation. This suggests that p110δ plays an important role in B cell function. Bruton's tyrosine kinase (Btk) is another kinase that plays a crucial role in B cell function. Btk is described in Vetri et al., *Nature*, 361: 226-233 (1993) and Tsukata et al., *Cell*, 72: 279-290 (1993). Btk is preferentially expressed in B cells, mast cells and platelets. Btk is an X-linked gene, and defects in Btk activity results in X-linked agammaglobulinemia (XLA). Primary symptoms of XLA are low numbers of peripheral B cells and cosequently low antibody titer. Similar to the defects in human B cells, in mice loss of Btk results in defective B cell development and function. A remarkably similar defect in B cell compartment was observed in PI kinase subunit p85 knock out mice. Since p85 is an essential component of class IA PI3 kinases, in addition to Btk, PI3 kinase(s) are apparently required for B cell function. Since Btk has an SH3 domain, it is contemplated that p110δ interacts with the SH3 domain of Btk through its polyproline sequence.

30 In neutrophils and B cells therefore, it is contemplated that the polyproline sequence of p110δ is involved in interactions with the SH3 domains of p47-phox and Btk respectively. However, in addition to interacting with other proteins, the polyproline sequence of p110δ can interact with components of PI3 kinase itself. It may be recalled that class IA kinases consist of a catalytic subunit (p10) and a



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regulatory subunit (p85). P85 is described in Escobedo et al., *Cell*, 65: 75-82 (1991); Skolnik et al., *Cell*, 65: 83-90 (1991) and Otsu et al., *Cell*, 65: 91-104 (1991).

Association of p85 is essential for the catalytic activity of p110. Though the regions responsible for the binding of p85 subunit to p110 subunit have been mapped, it is contemplated that the p110 subunit of PI3 kinase delta provides an additional binding site to the SH3 domain of p85 through its unique polyproline region, thereby further enhancing the interaction between p85 and p110δ.

In summary the above example illustrates binding of the proline-rich sequence of p110δ to SH3 domains of other proteins (e.g., p47-phox, Btk) or a component of PI3 kinase itself (e.g., p85) and regulation of cellular activity.

All publications and patent documents cited in this specification are incorporated herein by reference for all that they disclose.

While the present invention has been described with specific reference to certain preferred embodiments for purposes of clarity and understanding, it will be apparent to the skilled artisan that further changes and modifications may be practiced within the scope of the invention as it is defined in the claims set forth below. Accordingly, no limitations should be placed on the invention other than those specifically recited in the claims.

**WHAT IS CLAIMED IS:**

1. A method of identifying a modulator of p110 $\delta$  binding to LASP-1, comprising the steps of:
  - (a) providing a p110 $\delta$  polypeptide and a LASP-1 polypeptide having specific binding affinity for one another;
  - (b) measuring binding between the p110 $\delta$  polypeptide and the LASP-1 polypeptide in the presence and absence of a test compound; and
  - (c) identifying the test compound as a modulator of p110 $\delta$  binding to LASP-1 when a different amount of binding of the p110 $\delta$  polypeptide to the LASP-1 polypeptide is detected in the presence of the test compound than in the absence of the test compound.
2. A method according to Claim 1, wherein the p110 $\delta$  polypeptide comprises the proline rich region of p110 $\delta$ .
3. A method according to Claim 1, wherein the LASP-1 polypeptide comprises the SH3 region of LASP-1.
4. A method according to Claim 1, wherein the p110 $\delta$  polypeptide is provided as a fusion protein.
5. A method according to Claim 1, wherein the LASP-1 polypeptide is provided as a fusion protein.
6. A method according to Claim 1, wherein the p110 $\delta$  polypeptide is expressed by a host cell.
7. A method according to Claim 1, wherein the LASP-1 polypeptide is expressed by a host cell.
8. A method according to Claim 1, wherein the p110 $\delta$  polypeptide and the LASP-1 polypeptide are recombinantly coexpressed by a host cell.

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9. A method according to Claim 1, wherein the modulator inhibits binding of p110 $\delta$  to LASP-1.
10. A method according to Claim 1, wherein the modulator enhances binding of p110 $\delta$  to LASP-1.
11. A compound having activity as a modulator of interaction between p110 $\delta$  and LASP-1, wherein the compound is identified according to the method of Claim 1.
12. A compound according to Claim 11, wherein the compound inhibits binding of p110 $\delta$  to LASP-1.
13. A method of treating a disease state characterized by undesirable or excessive activity of PI3K $\delta$ , comprising administering to a subject in need thereof a compound according to Claim 11 or a derived analog thereof in an amount effective to inhibit interaction of p110 $\delta$  with LASP-1.
14. A method according to Claim 13, wherein the modulator inhibits binding of p110 $\delta$  to LASP-1.
15. A method of modulating p110 $\delta$  binding to LASP-1, comprising the step of contacting p110 $\delta$  or LASP-1 with a modulator of p110 $\delta$  binding to LASP-1.
16. A method according to Claim 15, wherein the modulator inhibits binding of p110 $\delta$  to LASP-1.
17. A method of treating a disease state associated with p110 $\delta$  binding to LASP-1, comprising the step of administering to a subject in need thereof an effective amount of a modulator of p110 $\delta$  binding to LASP-1.

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18. A method according to Claim 17, wherein the modulator inhibits binding of p110 $\delta$  to LASP-1.

19. A method of identifying a modulator of p110 $\delta$  binding to a polypeptide comprising an SH3 domain, comprising the steps of:

(a) providing a p110 $\delta$  polypeptide and an SH3 domain-containing polypeptide having specific binding affinity for one another;

(b) measuring binding between the p110 $\delta$  polypeptide and the SH3 domain-containing polypeptide in the presence and absence of a test compound; and

(c) identifying the test compound as a modulator of p110 $\delta$  binding to the SH3 domain-containing polypeptide when a different amount of binding of the p110 $\delta$  polypeptide to the SH3 domain-containing polypeptide is detected in the presence of the test compound than in the absence of the test compound.

20. A method according to Claim 19, wherein the p110 $\delta$  polypeptide is provided as a fusion protein.

21. A method according to Claim 19, wherein the SH3 domain-containing polypeptide is provided as a fusion protein.

22. A method according to Claim 19, wherein the p110 $\delta$  polypeptide is expressed by a host cell.

23. A method according to Claim 19, wherein the SH3 domain-containing polypeptide is expressed by a host cell.

24. A method according to Claim 19, wherein the p110 $\delta$  polypeptide and the SH3 domain-containing polypeptide are recombinantly coexpressed by a host cell.

25. A method according to Claim 19, wherein the modulator inhibits binding of p110 $\delta$  to the SH3 domain-containing polypeptide.

26. A method according to Claim 19, wherein the modulator enhances binding of p110 $\delta$  to the SH3 domain-containing polypeptide.

27. A compound having activity as a modulator of interaction between p110 $\delta$  and an SH3 domain-containing polypeptide, wherein the compound is identified according to the method of Claim 19.

28. A compound according to Claim 28, wherein the compound inhibits binding of p110 $\delta$  to the SH3 domain-containing polypeptide.

29. A method of treating a disease state characterized by undesirable or excessive activity of PI3K $\delta$ , comprising administering to a subject in need thereof a compound according to Claim 27.

30. A method according to Claim 29, wherein the compound inhibits binding of p110 $\delta$  to the SH3 domain-containing polypeptide.

31. A method of modulating p110 $\delta$  binding to an SH3 domain-containing polypeptide, comprising the step of contacting p110 $\delta$  or the SH3 domain-containing polypeptide with a modulator of p110 $\delta$  binding to the SH3 domain-containing polypeptide.

32. A method according to Claim 31, wherein the modulator inhibits binding of p110 $\delta$  to the SH3 domain-containing polypeptide.

33. A method of treating a disease state associated with p110 $\delta$  binding to an SH3 domain-containing polypeptide, comprising the step of administering to a subject in need thereof an effective amount of a modulator of p110 $\delta$  binding to the SH3 domain-containing polypeptide.

34. A method according to Claim 33, wherein the modulator inhibits binding of p110 $\delta$  to the SH3 domain-containing polypeptide.

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35. The method of claim 19, 29, 31 or 33 wherein the SH3 domain-containing polypeptide is p85.

36. The method of claim 19, 29, 31 or 33 wherein the SH3 domain-containing polypeptide is p47-phox.

37. The method of claim 19, 29, 31 or 33 wherein the SH3 domain-containing polypeptide is Btk.

## SEQUENCE LISTING

&lt;110&gt; Sadhu, Chanchal

&lt;120&gt; PHOSPHATIDYL INOSITOL 3-KINASE DELTA BINDING PARTNER

&lt;130&gt; 36828PCT

&lt;140&gt;

&lt;141&gt;

&lt;160&gt; 18

&lt;170&gt; PatentIn Ver. 2.1

&lt;210&gt; 1

&lt;211&gt; 3868

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (197)..(3331)

<223> Human p110 delta cDNA (GenBank Accession No.  
NM\_005026)

&lt;400&gt; 1

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ggcggagggg gctttgctgg tctttcttgg actattccag agaggacaac tgtcatctgg 180
gaagtaacaa cgcagg atg ccc cct ggg gtg gac tgc ccc atg gaa ttc tgg 232
               Met Pro Pro Gly Val Asp Cys Pro Met Glu Phe Trp
                1             5             10

acc aag gag gag aat cag agc gtt gtg gtt gac ttc ctg ctg ccc aca 280
Thr Lys Glu Glu Asn Gln Ser Val Val Val Asp Phe Leu Leu Pro Thr
                15             20             25

ggg gtc tac ctg aac ttc cct gtg tcc cgc aat gcc aac ctc agc acc 328
Gly Val Tyr Leu Asn Phe Pro Val Ser Arg Asn Ala Asn Leu Ser Thr
                30             35             40

atc aag cag ctg ctg tgg cac cgc gcc cag tat gag ccg ctc ttc cac 376
Ile Lys Gln Leu Leu Trp His Arg Ala Gln Tyr Glu Pro Leu Phe His
                45             50             55             60

atg ctc agt ggc ccc gag gcc tat gtg ttc acc tgc atc aac cag aca 424
Met Leu Ser Gly Pro Glu Ala Tyr Val Phe Thr Cys Ile Asn Gln Thr
                65             70             75

gcg gag cag caa gag ctg gag gac gag caa cgg cgt ctg tgt gac gtg 472
Ala Glu Gln Gln Glu Leu Glu Asp Glu Gln Arg Arg Leu Cys Asp Val
                80             85             90

cag ccc ttc ctg ccc gtc ctg cgc ctg gtg gcc cgt gag ggc gac cgc 520
Gln Pro Phe Leu Pro Val Leu Arg Leu Val Ala Arg Glu Gly Asp Arg
                95             100             105

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-2-

gtg aag aag ctc atc aac tca cag atc agc ctc ctc atc ggc aaa ggc	568
Val Lys Lys Leu Ile Asn Ser Gln Ile Ser Leu Leu Ile Gly Lys Gly	
110 115 120	
ctc cac gag ttt gac tcc ttg tgc gac cca gaa gtg aac gac ttt cgc	616
Leu His Glu Phe Asp Ser Leu Cys Asp Pro Glu Val Asn Asp Phe Arg	
125 130 135 140	
gcc aag atg tgc caa ttc tgc gag gag gcg gcc gcc cgc cgg cag cag	664
Ala Lys Met Cys Gln Phe Cys Glu Glu Ala Ala Ala Arg Arg Gln Gln	
145 150 155	
ctg ggc tgg gag gcc tgg ctg cag tac agt ttc ccc ctg cag ctg gag	712
Leu Gly Trp Glu Ala Trp Leu Gln Tyr Ser Phe Pro Leu Gln Leu Glu	
160 165 170	
ccc tcg gct caa acc tgg ggg cct ggt acc ctg cgg ctc ccg aac cgg	760
Pro Ser Ala Gln Thr Trp Gly Pro Gly Thr Leu Arg Leu Pro Asn Arg	
175 180 185	
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Ala Leu Leu Val Asn Val Lys Phe Glu Gly Ser Glu Glu Ser Phe Thr	
190 195 200	
ttc cag gtg tcc acc aag gac gtg ccg ctg gcg ctg atg gcc tgt gcc	856
Phe Gln Val Ser Thr Lys Asp Val Pro Leu Ala Leu Met Ala Cys Ala	
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Glu Asp Tyr Thr Leu Gln Val Asn Gly Arg His Glu Tyr Leu Tyr Gly	
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Ser Tyr Pro Leu Cys Gln Phe Gln Tyr Ile Cys Ser Cys Leu His Ser	
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270 275 280	
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Trp Ser Leu Glu Gln Pro Phe Arg Ile Glu Leu Ile Gln Gly Ser Lys	
320 325 330	
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Val Asn Ala Asp Glu Arg Met Lys Leu Val Val Gln Ala Gly Leu Phe	
335 340 345	



-3-

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His Gly Asn Glu Met Leu Cys Lys Thr Val Ser Ser Ser Glu Val Ser	
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gtg tgc tcg gag ccc gtg tgg aag cag cgg ctg gag ttc gac atc aac	1336
Val Cys Ser Glu Pro Val Trp Lys Gln Arg Leu Glu Phe Asp Ile Asn	
365 370 375 380	
atc tgc gac ctg ccc cgc atg gcc cgt ctc tgc ttt gcg ctg tac gcc	1384
Ile Cys Asp Leu Pro Arg Met Ala Arg Leu Cys Phe Ala Leu Tyr Ala	
385 390 395	
gtg atc gag aaa gcc aag aag gct cgc tcc acc aag aag aag tcc aag	1432
Val Ile Glu Lys Ala Lys Lys Ala Arg Ser Thr Lys Lys Lys Ser Lys	
400 405 410	
aag gcg gac tgc ccc att gcc tgg gcc aac ctc atg ctg ttt gac tac	1480
Lys Ala Asp Cys Pro Ile Ala Trp Ala Asn Leu Met Leu Phe Asp Tyr	
415 420 425	
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Lys Asp Gln Leu Lys Thr Gly Glu Arg Cys Leu Tyr Met Trp Pro Ser	
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Val Pro Asp Glu Lys Gly Glu Leu Leu Asn Pro Thr Gly Thr Val Arg	
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Ser Asn Pro Asn Thr Asp Ser Ala Ala Leu Leu Ile Cys Leu Pro	
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Glu His Glu Lys Asp Leu Val Trp Lys Leu Arg His Glu Val Gln Glu	
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His Phe Pro Glu Ala Leu Ala Arg Leu Leu Leu Val Thr Lys Trp Asn	
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Lys His Glu Asp Val Ala Gln Met Leu Tyr Leu Leu Cys Ser Trp Pro	
560 565 570	
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Val Ile Gln Gln Gly Lys Thr Asn Asn Ser Glu Lys Phe Glu Arg Phe	
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Gly Lys Thr Glu Glu Glu Ala Leu Lys His Phe Arg Val Lys Phe Asn	
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 Glu Thr Cys Lys Met Thr Leu Asn Met Lys Asn Tyr Lys Gly Tyr Glu  
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 Lys Lys Pro Tyr Cys Asn Ala His Tyr Pro Lys Gln Ser Phe Thr Met  
 50 55 60  
 Val Ala Asp Thr Pro Glu Asn Leu Arg Leu Lys Gln Gln Ser Glu Leu  
 65 70 75 80  
 Gln Ser Gln Val Arg Tyr Lys Glu Glu Phe Glu Lys Asn Lys Gly Lys  
 85 90 95  
 Gly Phe Ser Val Val Ala Asp Thr Pro Glu Leu Gln Arg Ile Lys Lys  
 100 105 110  
 Thr Gln Asp Gln Ile Ser Asn Ile Lys Tyr His Glu Glu Phe Glu Lys  
 115 120 125  
 Ser Arg Met Gly Pro Ser Gly Gly Glu Gly Met Glu Pro Glu Arg Arg  
 130 135 140  
 Asp Ser Gln Asp Gly Ser Ser Tyr Arg Arg Pro Leu Glu Gln Gln Gln  
 145 150 155 160

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Pro His His Ile Pro Thr Ser Ala Pro Val Tyr Gln Gln Pro Gln Gln  
 165 170 175

Gln Pro Val Ala Gln Ser Tyr Gly Gly Tyr Lys Glu Pro Ala Ala Pro  
 180 185 190

Val Ser Ile Gln Arg Ser Ala Pro Gly Gly Gly Gly Lys Arg Tyr Arg  
 195 200 205

Ala Val Tyr Asp Tyr Ser Ala Ala Asp Glu Asp Glu Val Ser Phe Gln  
 210 215 220

Asp Gly Asp Thr Ile Val Asn Val Gln Gln Ile Asp Asp Gly Trp Met  
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Tyr Val Glu Ala Ile  
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 His Leu Met Pro Pro Arg Ile Leu Val Glu Cys Leu Leu Pro Asn Gly  
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atg ata gtg act tta gaa tgc ctc cgt gag gct aca tta gta act ata 147  
 Met Ile Val Thr Leu Glu Cys Leu Arg Glu Ala Thr Leu Val Thr Ile  
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aag cat gaa cta ttt aaa gaa gca aga aaa tac cct ctc cat caa ctt 195  
 Lys His Glu Leu Phe Lys Glu Ala Arg Lys Tyr Pro Leu His Gln Leu  
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ctt caa gat gaa tct tct tac att ttc gta agt gtt acc caa gaa gca 243  
 Leu Gln Asp Glu Ser Ser Tyr Ile Phe Val Ser Val Thr Gln Glu Ala  
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gaa agg gaa gaa ttt ttt gat gaa aca aga cga ctt tgt gat ctt cgg 291  
 Glu Arg Glu Glu Phe Phe Asp Glu Thr Arg Arg Leu Cys Asp Leu Arg  
 80 85 90

ctt ttt caa cca ttt tta aaa gta att gaa cca gta ggc aac cgt gaa 339  
 Leu Phe Gln Pro Phe Leu Lys Val Ile Glu Pro Val Gly Asn Arg Glu  
 95 100 105

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Glu Lys Ile Leu Asn Arg Glu Ile Gly Phe Ala Ile Gly Met Pro Val	
110 115 120 125	
tgc gaa ttt gat atg gtt aaa gat cct gaa gta cag gac ttc cga aga	435
Cys Glu Phe Asp Met Val Lys Asp Pro Glu Val Gln Asp Phe Arg Arg	
130 135 140	
aat att ctt aat gtt tgt aaa gaa gct gtg gat ctt agg gat ctt aat	483
Asn Ile Leu Asn Val Cys Lys Glu Ala Val Asp Leu Arg Asp Leu Asn	
145 150 155	
tca cct cat agt aga gca atg tat gtc tat ccg cca cat gta gaa tct	531
Ser Pro His Ser Arg Ala Met Tyr Val Tyr Pro Pro His Val Glu Ser	
160 165 170	
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Ser Pro Glu Leu Pro Lys His Ile Tyr Asn Lys Leu Asp Arg Gly Gln	
175 180 185	
ata ata gtg gtg att tgg gta ata gtt tct cca aat aat gac aag cag	627
Ile Ile Val Val Ile Trp Val Ile Val Ser Pro Asn Asn Asp Lys Gln	
190 195 200 205	
aag tat act ctg aaa atc aac cat gac tgt gtg cca gaa caa gta att	675
Lys Tyr Thr Leu Lys Ile Asn His Asp Cys Val Pro Glu Gln Val Ile	
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Ala Glu Ala Ile Arg Lys Lys Thr Arg Ser Met Leu Leu Ser Ser Glu	
225 230 235	
caa tta aaa ctc tgt gtt tta gaa tat cag ggc aag tac att tta aaa	771
Gln Leu Lys Leu Cys Val Leu Glu Tyr Gln Gly Lys Tyr Ile Leu Lys	
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Val Cys Gly Cys Asp Glu Tyr Phe Leu Glu Lys Tyr Pro Leu Ser Gln	
255 260 265	
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Tyr Lys Tyr Ile Arg Ser Cys Ile Met Leu Gly Arg Met Pro Asn Leu	
270 275 280 285	
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Lys Met Met Ala Lys Glu Ser Leu Tyr Ser Gln Leu Pro Met Asp Cys	
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ttt aca atg cca tct tat tcc aga cgc att tcc aca gct aca cca tat	963
Phe Thr Met Pro Ser Tyr Ser Arg Arg Ile Ser Thr Ala Thr Pro Tyr	
305 310 315	
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Met Asn Gly Glu Thr Ser Thr Lys Ser Leu Trp Val Ile Asn Arg Ala	
320 325 330	
ctc aga ata aaa att ctt tgt gca acc tac gtg aat cta aat att cga	1059
Leu Arg Ile Lys Ile Leu Cys Ala Thr Tyr Val Asn Leu Asn Ile Arg	
335 340 345	

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Asp Ile Asp Lys Ile Tyr Val Arg Thr Gly Ile Tyr His Gly Gly Glu	
350 355 360 365	
ccc tta tgt gac aat gtg aac act caa aga gta cct tgt tcc aat ccc	1155
Pro Leu Cys Asp Asn Val Asn Thr Gln Arg Val Pro Cys Ser Asn Pro	
370 375 380	
agg tgg aat gaa tgg ctg aat tat gat ata tac att cct gat ctt cct	1203
Arg Trp Asn Glu Trp Leu Asn Tyr Asp Ile Tyr Ile Pro Asp Leu Pro	
385 390 395	
cgt gct gct cga ctt tgc ctt tcc att tgc tct gtt aaa ggc cga aag	1251
Arg Ala Ala Arg Leu Cys Leu Ser Ile Cys Ser Val Lys Gly Arg Lys	
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Gly Ala Lys Glu Glu His Cys Pro Leu Ala Trp Gly Asn Ile Asn Leu	
415 420 425	
ttt gat tac aca gac act cta gta tct gga aaa atg gct ttg aat ctt	1347
Phe Asp Tyr Thr Asp Thr Leu Val Ser Gly Lys Met Ala Leu Asn Leu	
430 435 440 445	
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Trp Pro Val Pro His Gly Leu Glu Asp Leu Leu Asn Pro Ile Gly Val	
450 455 460	
act gga tca aat cca aat aaa gaa act cca tgc tta gag ttg gag ttt	1443
Thr Gly Ser Asn Pro Asn Lys Glu Thr Pro Cys Leu Glu Leu Glu Phe	
465 470 475	
gac tgg ttc agc agt gtg gta aag ttc cca gat atg tca gtg att gaa	1491
Asp Trp Phe Ser Ser Val Val Lys Phe Pro Asp Met Ser Val Ile Glu	
480 485 490	
gag cat gcc aat tgg tct gta tcc cga gaa gca gga ttt agc tat tcc	1539
Glu His Ala Asn Trp Ser Val Ser Arg Glu Ala Gly Phe Ser Tyr Ser	
495 500 505	
cac gca gga ctg agt aac aga cta gct aga gac aat gaa tta agg gaa	1587
His Ala Gly Leu Ser Asn Arg Leu Ala Arg Asp Asn Glu Leu Arg Glu	
510 515 520 525	
aat gac aaa gaa cag ctc aaa gca att tct aca cga gat cct ctc tct	1635
Asn Asp Lys Glu Gln Leu Lys Ala Ile Ser Thr Arg Asp Pro Leu Ser	
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Glu Ile Thr Glu Gln Glu Lys Asp Phe Leu Trp Ser His Arg His Tyr	
545 550 555	
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Cys Val Thr Ile Pro Glu Ile Leu Pro Lys Leu Leu Leu Ser Val Lys	
560 565 570	
tgg aat tct aga gat gaa gta gcc cag atg tat tgc ttg gta aaa gat	1779
Trp Asn Ser Arg Asp Glu Val Ala Gln Met Tyr Cys Leu Val Lys Asp	
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590 595 600 605	
tac cca gat cct atg gtt cga ggt ttt gct gtt cgg tgc ttg gaa aaa	1875
Tyr Pro Asp Pro Met Val Arg Gly Phe Ala Val Arg Cys Leu Glu Lys	
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Tyr Leu Thr Asp Asp Lys Leu Ser Gln Tyr Leu Ile Gln Leu Val Gln	
625 630 635	
gtc cta aaa tat gaa caa tat ttg gat aac ttg ctt gtg aga ttt tta	1971
Val Leu Lys Tyr Glu Gln Tyr Leu Asp Asn Leu Leu Val Arg Phe Leu	
640 645 650	
ctg aag aaa gca ttg act aat caa agg att ggg cac ttt ttc ttt tgg	2019
Leu Lys Lys Ala Leu Thr Asn Gln Arg Ile Gly His Phe Phe Phe Trp	
655 660 665	
cat tta aaa tct gag atg cac aat aaa aca gtt agc cag agg ttt ggc	2067
His Leu Lys Ser Glu Met His Asn Lys Thr Val Ser Gln Arg Phe Gly	
670 675 680 685	
ctg ctt ttg gag tcc tat tgt cgt gca tgt ggg atg tat ttg aag cac	2115
Leu Leu Leu Glu Ser Tyr Cys Arg Ala Cys Gly Met Tyr Leu Lys His	
690 695 700	
ctg aat agg caa gtc gag gca atg gaa aag ctc att aac tta act gac	2163
Leu Asn Arg Gln Val Glu Ala Met Glu Lys Leu Ile Asn Leu Thr Asp	
705 710 715	
att ctc aaa cag gag agg aag gat gaa aca caa aag gta cag atg aag	2211
Ile Leu Lys Gln Glu Arg Lys Asp Glu Thr Gln Lys Val Gln Met Lys	
720 725 730	
ttt tta gtt gag caa atg agg cga cca gat ttc atg gat gcc cta cag	2259
Phe Leu Val Glu Gln Met Arg Arg Pro Asp Phe Met Asp Ala Leu Gln	
735 740 745	
ggc ttg ctg tct cct cta aac cct gct cat caa cta gga aac ctc agg	2307
Gly Leu Leu Ser Pro Leu Asn Pro Ala His Gln Leu Gly Asn Leu Arg	
750 755 760 765	
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Leu Lys Glu Cys Arg Ile Met Ser Ser Ala Lys Arg Pro Leu Trp Leu	
770 775 780	
aat tgg gag aac cca gac atc atg tca gag tta ctg ttt cag aac aat	2403
Asn Trp Glu Asn Pro Asp Ile Met Ser Glu Leu Leu Phe Gln Asn Asn	
785 790 795	
gag atc atc ttt aaa aat ggg gat gat tta cgg caa gat atg cta aca	2451
Glu Ile Ile Phe Lys Asn Gly Asp Asp Leu Arg Gln Asp Met Leu Thr	
800 805 810	
ctt caa att att cgt att atg gaa aat atc tgg caa aat caa ggt ctt	2499
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gga ctt att gag gtg gtg cga aat tct cac act att atg caa att cag	2595
Gly Leu Ile Glu Val Val Arg Asn Ser His Thr Ile Met Gln Ile Gln	
850 855 860	
tgc aaa ggc ggc ttg aaa ggt gca ctg cag ttc aac agc cac aca cta	2643
Cys Lys Gly Gly Leu Lys Gly Ala Leu Gln Phe Asn Ser His Thr Leu	
865 870 875	
cat cag tgg ctc aaa gac aag aac aaa gga gaa ata tat gat gca gcc	2691
His Gln Trp Leu Lys Asp Lys Asn Lys Gly Glu Ile Tyr Asp Ala Ala	
880 885 890	
att gac ctg ttt aca cgt tca tgt gct gga tac tgt gta gct acc ttc	2739
Ile Asp Leu Phe Thr Arg Ser Cys Ala Gly Tyr Cys Val Ala Thr Phe	
895 900 905	
att ttg gga att gga gat cgt cac aat agt aac atc atg gtg aaa gac	2787
Ile Leu Gly Ile Gly Asp Arg His Asn Ser Asn Ile Met Val Lys Asp	
910 915 920 925	
gat gga caa ctg ttt cat ata gat ttt gga cac ttt ttg gat cac aag	2835
Asp Gly Gln Leu Phe His Ile Asp Phe Gly His Phe Leu Asp His Lys	
930 935 940	
aag aaa aaa ttt ggt tat aaa cga gaa cgt gtg cca ttt gtt ttg aca	2883
Lys Lys Lys Phe Gly Tyr Lys Arg Glu Arg Val Pro Phe Val Leu Thr	
945 950 955	
cag gat ttc tta ata gtg att agt aaa gga gcc caa gaa tgc aca aag	2931
Gln Asp Phe Leu Ile Val Ile Ser Lys Gly Ala Gln Glu Cys Thr Lys	
960 965 970	
aca aga gaa ttt gag agg ttt cag gag atg tgt tac aag gct tat cta	2979
Thr Arg Glu Phe Glu Arg Phe Gln Glu Met Cys Tyr Lys Ala Tyr Leu	
975 980 985	
gct att cga cag cat gcc aat ctc ttc ata aat ctt ttc tca atg atg	3027
Ala Ile Arg Gln His Ala Asn Leu Phe Ile Asn Leu Phe Ser Met Met	
990 995 1000 1005	
ctt ggc tct gga atg cca gaa cta caa tct ttt gat gac att gca tac	3075
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1010 1015 1020	
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Ile Arg Lys Thr Leu Ala Leu Asp Lys Thr Glu Gln Glu Ala Leu Glu	
1025 1030 1035	
tat ttc atg aaa caa atg aat gat gca cat cat ggt ggc tgg aca aca	3171
Tyr Phe Met Lys Gln Met Asn Asp Ala His His Gly Gly Trp Thr Thr	
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aaa atg gat tgg atc ttc cac aca att aaa cag cat gca ttg aac tga	3219
Lys Met Asp Trp Ile Phe His Thr Ile Lys Gln His Ala Leu Asn	
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 Thr Leu Glu Cys Leu Arg Glu Ala Thr Leu Val Thr Ile Lys His Glu  
                     35                    40                    45  
 Leu Phe Lys Glu Ala Arg Lys Tyr Pro Leu His Gln Leu Leu Gln Asp  
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 Glu Ser Ser Tyr Ile Phe Val Ser Val Thr Gln Glu Ala Glu Arg Glu  
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 Glu Phe Phe Asp Glu Thr Arg Arg Leu Cys Asp Leu Arg Leu Phe Gln  
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 Pro Phe Leu Lys Val Ile Glu Pro Val Gly Asn Arg Glu Glu Lys Ile  
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 Asp Met Val Lys Asp Pro Glu Val Gln Asp Phe Arg Arg Asn Ile Leu  
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 Ser Arg Ala Met Tyr Val Tyr Pro Pro His Val Glu Ser Ser Pro Glu  
                     165                    170                    175  
 Leu Pro Lys His Ile Tyr Asn Lys Leu Asp Arg Gly Gln Ile Ile Val  
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 Val Ile Trp Val Ile Val Ser Pro Asn Asn Asp Lys Gln Lys Tyr Thr  
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 Leu Lys Ile Asn His Asp Cys Val Pro Glu Gln Val Ile Ala Glu Ala  
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 Ile Arg Lys Lys Thr Arg Ser Met Leu Leu Ser Ser Glu Gln Leu Lys  
                     225                    230                    235                    240  
 Leu Cys Val Leu Glu Tyr Gln Gly Lys Tyr Ile Leu Lys Val Cys Gly  
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 Ile Arg Ser Cys Ile Met Leu Gly Arg Met Pro Asn Leu Lys Met Met  
 275 280 285  
 Ala Lys Glu Ser Leu Tyr Ser Gln Leu Pro Met Asp Cys Phe Thr Met  
 290 295 300  
 Pro Ser Tyr Ser Arg Arg Ile Ser Thr Ala Thr Pro Tyr Met Asn Gly  
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 Glu Thr Ser Thr Lys Ser Leu Trp Val Ile Asn Arg Ala Leu Arg Ile  
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 Lys Ile Leu Cys Ala Thr Tyr Val Asn Leu Asn Ile Arg Asp Ile Asp  
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 Glu Trp Leu Asn Tyr Asp Ile Tyr Ile Pro Asp Leu Pro Arg Ala Ala  
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 565 570 575  
 Arg Asp Glu Val Ala Gln Met Tyr Cys Leu Val Lys Asp Trp Pro Pro  
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 Pro Met Val Arg Gly Phe Ala Val Arg Cys Leu Glu Lys Tyr Leu Thr  
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 Tyr Glu Gln Tyr Leu Asp Asn Leu Leu Val Arg Phe Leu Leu Lys Lys  
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 Ser Glu Met His Asn Lys Thr Val Ser Gln Arg Phe Gly Leu Leu Leu  
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 Ser Pro Leu Asn Pro Ala His Gln Leu Gly Asn Leu Arg Leu Lys Glu  
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 Asp Phe Leu Leu Pro Thr Gly Ile Tyr Ile Gln Leu Glu Val Pro Arg  
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 gaa gct acc att tct tat att aag cag atg tta tgg aag caa gtt cac 192  
 Glu Ala Thr Ile Ser Tyr Ile Lys Gln Met Leu Trp Lys Gln Val His  
 50 55 60  
 aat tac cca atg ttc aac ctc ctt atg gat att gac tcc tat atg ttt 240  
 Asn Tyr Pro Met Phe Asn Leu Leu Met Asp Ile Asp Ser Tyr Met Phe  
 65 70 75 80

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gca tgt gtg aat cag act gct gta tat gag gag ctt gaa gat gaa aca	288
Ala Cys Val Asn Gln Thr Ala Val Tyr Glu Glu Leu Glu Asp Glu Thr	
85 90 95	
cga aga ctc tgt gat gtc aga cct ttt ctt cca gtt ctc aaa tta gtg	336
Arg Arg Leu Cys Asp Val Arg Pro Phe Leu Pro Val Leu Lys Leu Val	
100 105 110	
aca aga agt tgt gac cca ggg gaa aaa tta gac tca aaa att gga gtc	384
Thr Arg Ser Cys Asp Pro Gly Glu Lys Leu Asp Ser Lys Ile Gly Val	
115 120 125	
ctt ata gga aaa ggt ctg cat gaa ttt gat tcc ttg aag gat cct gaa	432
Leu Ile Gly Lys Gly Leu His Glu Phe Asp Ser Leu Lys Asp Pro Glu	
130 135 140	
gta aat gaa ttt cga aga aaa atg cgc aaa ttc agc gag gaa aaa atc	480
Val Asn Glu Phe Arg Arg Lys Met Arg Lys Phe Ser Glu Glu Lys Ile	
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Leu Ser Leu Val Gly Leu Ser Trp Met Asp Trp Leu Lys Gln Thr Tyr	
165 170 175	
cca cca gag cat gaa cca tcc atc cct gaa aac tta gaa gat aaa ctt	576
Pro Pro Glu His Glu Pro Ser Ile Pro Glu Asn Leu Glu Asp Lys Leu	
180 185 190	
tat ggg gga aag ctc atc gta gct gtt cat ttt gaa aac tgc cag gac	624
Tyr Gly Gly Lys Leu Ile Val Ala Val His Phe Glu Asn Cys Gln Asp	
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gtg ttt agc ttt caa gtg tct cct aat atg aat cct atc aaa gta aat	672
Val Phe Ser Phe Gln Val Ser Pro Asn Met Asn Pro Ile Lys Val Asn	
210 215 220	
gaa ttg gca atc caa aaa cgt ttg act att cat ggg aag gaa gat gaa	720
Glu Leu Ala Ile Gln Lys Arg Leu Thr Ile His Gly Lys Glu Asp Glu	
225 230 235 240	
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Val Ser Pro Tyr Asp Tyr Val Leu Gln Val Ser Gly Arg Val Glu Tyr	
245 250 255	
gtt ttt ggt gat cat cca cta att cag ttc cag tat atc cgg aac tgt	816
Val Phe Gly Asp His Pro Leu Ile Gln Phe Gln Tyr Ile Arg Asn Cys	
260 265 270	
gtg atg aac aga gcc ctg ccc cat ttt ata ctt gtg gaa tgc tgc aag	864
Val Met Asn Arg Ala Leu Pro His Phe Ile Leu Val Glu Cys Cys Lys	
275 280 285	
atc aag aaa atg tat gaa caa gaa atg att gcc ata gag gct gcc ata	912
Ile Lys Lys Met Tyr Glu Gln Glu Met Ile Ala Ile Glu Ala Ala Ile	
290 295 300	
aat cga aat tca tct aat ctt cct ctt cca tta cca cca aag aaa aca	960
Asn Arg Asn Ser Ser Asn Leu Pro Leu Pro Leu Pro Pro Lys Lys Thr	
305 310 315 320	

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cga att att tct cat gtt tgg gaa aat aac aac cct ttc caa att gtc	1008
Arg Ile Ile Ser His Val Trp Glu Asn Asn Asn Pro Phe Gln Ile Val	
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Leu Val Lys Gly Asn Lys Leu Asn Thr Glu Glu Thr Val Lys Val His	
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gtc agg gct ggt ctt ttt cat ggt act gag ctc ctg tgt aaa acc atc	1104
Val Arg Ala Gly Leu Phe His Gly Thr Glu Leu Leu Cys Lys Thr Ile	
355 360 365	
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Val Ser Ser Glu Val Ser Gly Lys Asn Asp His Ile Trp Asn Glu Pro	
370 375 380	
ctg gaa ttt gat att aat att tgt gac tta cca aga atg gct cga tta	1200
Leu Glu Phe Asp Ile Asn Ile Cys Asp Leu Pro Arg Met Ala Arg Leu	
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tgt ttt gct gtt tat gca gtt ttg gat aaa gta aaa acg aag aaa tca	1248
Cys Phe Ala Val Tyr Ala Val Leu Asp Lys Val Lys Thr Lys Lys Ser	
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acg aaa act att aat ccc tct aaa tat cag acc atc agg aaa gct gga	1296
Thr Lys Thr Ile Asn Pro Ser Lys Tyr Gln Thr Ile Arg Lys Ala Gly	
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aaa gtg cat tat cct gta gcg tgg gta aat acg atg gtt ttt gac ttt	1344
Lys Val His Tyr Pro Val Ala Trp Val Asn Thr Met Val Phe Asp Phe	
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aaa gga caa ttg aga act gga gac ata ata tta cac agc tgg tct tca	1392
Lys Gly Gln Leu Arg Thr Gly Asp Ile Ile Leu His Ser Trp Ser Ser	
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Phe Pro Asp Glu Leu Glu Glu Met Leu Asn Pro Met Gly Thr Val Gln	
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Thr Asn Pro Tyr Thr Glu Asn Ala Thr Ala Leu His Val Lys Phe Pro	
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Glu Asn Lys Lys Gln Pro Tyr Tyr Tyr Pro Pro Phe Asp Lys Ile Ile	
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gaa aag gca gct gag att gca agc agt gat agt gct aat gtg tca agt	1584
Glu Lys Ala Ala Glu Ile Ala Ser Ser Asp Ser Ala Asn Val Ser Ser	
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Arg Gly Gly Lys Lys Phe Leu Pro Val Leu Lys Glu Ile Leu Asp Arg	
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Asp Pro Leu Ser Gln Leu Cys Glu Asn Glu Met Asp Leu Ile Trp Thr	
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ctg ctg tca atc aag tgg aat aaa ctt gag gat gtt gct cag ctt cag	1776
Leu Leu Ser Ile Lys Trp Asn Lys Leu Glu Asp Val Ala Gln Leu Gln	
580 585 590	
gcg ctg ctt cag att tgg cct aaa ctg ccc ccc cgg gag gcc cta gag	1824
Ala Leu Leu Gln Ile Trp Pro Lys Leu Pro Pro Arg Glu Ala Leu Glu	
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Gly Cys Leu Arg Gln Met Ser Asp Glu Glu Leu Ser Gln Tyr Leu Leu	
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Gln Leu Val Gln Val Leu Lys Tyr Glu Pro Phe Leu Asp Cys Ala Leu	
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tct aga ttc cta tta gaa aga gca ctt ggt aat cgg agg ata ggg cag	2016
Ser Arg Phe Leu Leu Glu Arg Ala Leu Gly Asn Arg Arg Ile Gly Gln	
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Phe Leu Phe Trp His Leu Arg Ser Glu Val His Ile Pro Ala Val Ser	
675 680 685	
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Val Gln Phe Gly Val Ile Leu Glu Ala Tyr Cys Arg Gly Ser Val Gly	
690 695 700	
cac atg aaa gtg ctt tct aag cag gtt gaa gca ctc aat aag tta aaa	2160
His Met Lys Val Leu Ser Lys Gln Val Glu Ala Leu Asn Lys Leu Lys	
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Thr Leu Asn Ser Leu Ile Lys Leu Asn Ala Val Lys Leu Asn Arg Ala	
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Lys Gly Lys Glu Ala Met His Thr Cys Leu Lys Gln Ser Ala Tyr Arg	
740 745 750	
gaa gcc ctc tct gac ctg cag tca ccc ctg aac cca tgt gtt atc ctc	2304
Glu Ala Leu Ser Asp Leu Gln Ser Pro Leu Asn Pro Cys Val Ile Leu	
755 760 765	
tca gaa ctc tat gtt gaa aag tgc aaa tac atg gat tcc aaa atg aag	2352
Ser Glu Leu Tyr Val Glu Lys Cys Lys Tyr Met Asp Ser Lys Met Lys	
770 775 780	
cct ttg tgg ctg gta tac aat aac aag gta ttt ggt gag gat tca gtt	2400
Pro Leu Trp Leu Val Tyr Asn Asn Lys Val Phe Gly Glu Asp Ser Val	
785 790 795 800	

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Gly	Val	Ile	Phe	Lys	Asn	Gly	Asp	Asp	Leu	Arg	Gln	Asp	Met	Leu	Thr	
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ctc	caa	atg	ttg	cgc	ttg	atg	gat	tta	ctc	tgg	aaa	gaa	gct	ggt	ttg	2496
Leu	Gln	Met	Leu	Arg	Leu	Met	Asp	Leu	Leu	Trp	Lys	Glu	Ala	Gly	Leu	
			820					825					830			
gat	ctt	cgg	atg	ttg	cct	tat	ggc	tgt	tta	gca	aca	gga	gat	cgc	tct	2544
Asp	Leu	Arg	Met	Leu	Pro	Tyr	Gly	Cys	Leu	Ala	Thr	Gly	Asp	Arg	Ser	
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Gly	Leu	Ile	Glu	Val	Val	Ser	Thr	Ser	Glu	Thr	Ile	Ala	Asp	Ile	Gln	
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Leu	Asn	Ser	Ser	Asn	Val	Ala	Ala	Ala	Ala	Ala	Phe	Asn	Lys	Asp	Ala	
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Leu	Leu	Asn	Trp	Leu	Lys	Glu	Tyr	Asn	Ser	Gly	Asp	Asp	Leu	Asp	Arg	
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gcc	att	gag	gaa	ttt	aca	ctg	tcc	tgt	gct	ggc	tac	tgt	gta	gct	tct	2736
Ala	Ile	Glu	Glu	Phe	Thr	Leu	Ser	Cys	Ala	Gly	Tyr	Cys	Val	Ala	Ser	
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Tyr	Val	Leu	Gly	Ile	Gly	Asp	Arg	His	Ser	Asp	Asn	Ile	Met	Val	Lys	
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Lys	Thr	Gly	Gln	Leu	Phe	His	Ile	Asp	Phe	Gly	His	Ile	Leu	Gly	Asn	
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Phe	Lys	Ser	Lys	Phe	Gly	Ile	Lys	Arg	Glu	Arg	Val	Pro	Phe	Ile	Leu	
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acc	tat	gat	ttc	atc	cat	gtc	att	caa	caa	gga	aaa	aca	gga	aat	aca	2928
Thr	Tyr	Asp	Phe	Ile	His	Val	Ile	Gln	Gln	Gly	Lys	Thr	Gly	Asn	Thr	
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gaa	aag	ttt	ggc	cgg	ttc	cgc	cag	tgt	tgt	gag	gat	gca	tat	ctg	att	2976
Glu	Lys	Phe	Gly	Arg	Phe	Arg	Gln	Cys	Cys	Glu	Asp	Ala	Tyr	Leu	Ile	
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Leu	Arg	Arg	His	Gly	Asn	Leu	Phe	Ile	Thr	Leu	Phe	Ala	Leu	Met	Leu	
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Thr	Ala	Gly	Leu	Pro	Glu	Leu	Thr	Ser	Val	Lys	Asp	Ile	Gln	Tyr	Leu	
	1010					1015					1020					
aag	gac	tct	ctt	gca	tta	ggg	aag	agt	gaa	gaa	gaa	gca	ctc	aaa	cag	3120
Lys	Asp	Ser	Leu	Ala	Leu	Gly	Lys	Ser	Glu	Glu	Glu	Ala	Leu	Lys	Gln	
1025					1030					1035				1040		

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 Phe Lys Gln Lys Phe Asp Glu Ala Leu Arg Glu Ser Trp Thr Thr Lys  
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gtg aac tgg atg gcc cac aca gtt cgg aaa gac tac aga tct taa 3213  
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 Asp Phe Leu Leu Pro Thr Gly Ile Tyr Ile Gln Leu Glu Val Pro Arg  
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 Glu Ala Thr Ile Ser Tyr Ile Lys Gln Met Leu Trp Lys Gln Val His  
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 Asn Tyr Pro Met Phe Asn Leu Leu Met Asp Ile Asp Ser Tyr Met Phe  
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 Ala Cys Val Asn Gln Thr Ala Val Tyr Glu Glu Leu Glu Asp Glu Thr  
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 Arg Arg Leu Cys Asp Val Arg Pro Phe Leu Pro Val Leu Lys Leu Val  
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 Thr Arg Ser Cys Asp Pro Gly Glu Lys Leu Asp Ser Lys Ile Gly Val  
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 Val Asn Glu Phe Arg Arg Lys Met Arg Lys Phe Ser Glu Glu Lys Ile  
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 Leu Ser Leu Val Gly Leu Ser Trp Met Asp Trp Leu Lys Gln Thr Tyr  
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 Pro Pro Glu His Glu Pro Ser Ile Pro Glu Asn Leu Glu Asp Lys Leu  
                     180                                    185                                    190  
 Tyr Gly Gly Lys Leu Ile Val Ala Val His Phe Glu Asn Cys Gln Asp  
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 Val Phe Ser Phe Gln Val Ser Pro Asn Met Asn Pro Ile Lys Val Asn  
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 Glu Leu Ala Ile Gln Lys Arg Leu Thr Ile His Gly Lys Glu Asp Glu  
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 Val Ser Pro Tyr Asp Tyr Val Leu Gln Val Ser Gly Arg Val Glu Tyr  
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Val	Met	Asn	Arg	Ala	Leu	Pro	His	Phe	Ile	Leu	Val	Glu	Cys	Cys	Lys		
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Ile	Lys	Lys	Met	Tyr	Glu	Gln	Glu	Met	Ile	Ala	Ile	Glu	Ala	Ala	Ile		
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Asn	Arg	Asn	Ser	Ser	Asn	Leu	Pro	Leu	Pro	Leu	Pro	Pro	Lys	Lys	Thr		
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Arg	Ile	Ile	Ser	His	Val	Trp	Glu	Asn	Asn	Asn	Pro	Phe	Gln	Ile	Val		
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Leu	Val	Lys	Gly	Asn	Lys	Leu	Asn	Thr	Glu	Glu	Thr	Val	Lys	Val	His		
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Val	Ser	Ser	Glu	Val	Ser	Gly	Lys	Asn	Asp	His	Ile	Trp	Asn	Glu	Pro		
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Cys	Phe	Ala	Val	Tyr	Ala	Val	Leu	Asp	Lys	Val	Lys	Thr	Lys	Lys	Ser		
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Lys	Gly	Gln	Leu	Arg	Thr	Gly	Asp	Ile	Ile	Leu	His	Ser	Trp	Ser	Ser		
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Glu	Asn	Lys	Lys	Gln	Pro	Tyr	Tyr	Tyr	Pro	Pro	Phe	Asp	Lys	Ile	Ile		
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-30-

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ctg ccc acc agc cag cgc aaa tgc aag agc ccc gaa acg gcg ctg ctg	497
Leu Pro Thr Ser Gln Arg Lys Cys Lys Ser Pro Glu Thr Ala Leu Leu	
45 50 55	
cac gtg gcc ggc cac ggc aac gtg gag cag atg aag gcc cag gtg tgg	545
His Val Ala Gly His Gly Asn Val Glu Gln Met Lys Ala Gln Val Trp	
60 65 70	
ctg cga gcg ctg gag acc agc gtg gcg gcg gac ttc tac cac cgg ctg	593
Leu Arg Ala Leu Glu Thr Ser Val Ala Ala Asp Phe Tyr His Arg Leu	
75 80 85 90	
gga ccg cat cac ttc ctc ctg ctc tat cag aag aag ggg cag tgg tac	641
Gly Pro His His Phe Leu Leu Leu Tyr Gln Lys Lys Gly Gln Trp Tyr	
95 100 105	
gag atc tac gac aag tac cag gtg gtg cag act ctg gac tgc ctg cgc	689
Glu Ile Tyr Asp Lys Tyr Gln Val Val Gln Thr Leu Asp Cys Leu Arg	
110 115 120	
tac tgg aag gcc acg cac cgg agc ccg ggc cag atc cac ctg gtg cag	737
Tyr Trp Lys Ala Thr His Arg Ser Pro Gly Gln Ile His Leu Val Gln	
125 130 135	
cgg cac ccg ccc tcc gag gag tcc caa gcc ttc cag cgg cag ctc acg	785
Arg His Pro Pro Ser Glu Glu Ser Gln Ala Phe Gln Arg Gln Leu Thr	
140 145 150	
gcg ctg att ggc tat gac gtc act gac gtc agc aac gtg cac gac gat	833
Ala Leu Ile Gly Tyr Asp Val Thr Asp Val Ser Asn Val His Asp Asp	
155 160 165 170	
gag ctg gag ttc acg cgc cgt ggc ttg gtg acc ccg cgc atg gcg gag	881
Glu Leu Glu Phe Thr Arg Arg Gly Leu Val Thr Pro Arg Met Ala Glu	
175 180 185	
gtg gcc agc cgc gac ccc aag ctc tac gcc atg cac ccg tgg gtg acg	929
Val Ala Ser Arg Asp Pro Lys Leu Tyr Ala Met His Pro Trp Val Thr	
190 195 200	
tcc aag ccc ctc ccg gag tac ctg tgg aag aag att gcc aac aac tgc	977
Ser Lys Pro Leu Pro Glu Tyr Leu Trp Lys Lys Ile Ala Asn Asn Cys	
205 210 215	
atc ttc atc gtc att cac cgc agc acc acc agc cag acc att aag gtc	1025
Ile Phe Ile Val Ile His Arg Ser Thr Thr Ser Gln Thr Ile Lys Val	
220 225 230	
tca ccc gac gac acc ccc ggc gcc atc ctg cag agc ttc ttc acc aag	1073
Ser Pro Asp Asp Thr Pro Gly Ala Ile Leu Gln Ser Phe Phe Thr Lys	
235 240 245 250	
atg gcc aag aag aaa tct ctg atg gat att ccc gaa agc caa agc gaa	1121
Met Ala Lys Lys Lys Ser Leu Met Asp Ile Pro Glu Ser Gln Ser Glu	
255 260 265	

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cag	gat	ttt	gtg	ctg	cgc	gtc	tgt	ggc	cgg	gat	gag	tac	ctg	gtg	ggc	1169
Gln	Asp	Phe	Val	Leu	Arg	Val	Cys	Gly	Arg	Asp	Glu	Tyr	Leu	Val	Gly	
			270					275					280			
gaa	acg	ccc	atc	aaa	aac	ttc	cag	tgg	gtg	agg	cac	tgc	ctc	aag	aac	1217
Glu	Thr	Pro	Ile	Lys	Asn	Phe	Gln	Trp	Val	Arg	His	Cys	Leu	Lys	Asn	
		285					290					295				
gga	gaa	gag	att	cac	gtg	gta	ctg	gac	acg	cct	cca	gac	ccg	gcc	cta	1265
Gly	Glu	Glu	Ile	His	Val	Val	Leu	Asp	Thr	Pro	Pro	Asp	Pro	Ala	Leu	
	300					305					310					
gac	gag	gtg	agg	aag	gaa	gag	tgg	ccg	ctg	gtg	gac	gac	tgc	acg	gga	1313
Asp	Glu	Val	Arg	Lys	Glu	Glu	Trp	Pro	Leu	Val	Asp	Asp	Cys	Thr	Gly	
315					320					325					330	
gtc	acc	ggc	tac	cat	gag	cag	ctt	acc	atc	cac	ggc	aag	gac	cac	gag	1361
Val	Thr	Gly	Tyr	His	Glu	Gln	Leu	Thr	Ile	His	Gly	Lys	Asp	His	Glu	
				335					340					345		
agt	gtg	ttc	acc	gtg	tcc	ctg	tgg	gac	tgc	gac	cgc	aag	ttc	agg	gtc	1409
Ser	Val	Phe	Thr	Val	Ser	Leu	Trp	Asp	Cys	Asp	Arg	Lys	Phe	Arg	Val	
			350					355					360			
aag	atc	aga	ggc	att	gat	atc	ccc	gtc	ctg	cct	cgg	aac	acc	gac	ctc	1457
Lys	Ile	Arg	Gly	Ile	Asp	Ile	Pro	Val	Leu	Pro	Arg	Asn	Thr	Asp	Leu	
		365					370					375				
aca	gtt	ttt	gta	gag	gca	aac	atc	cag	cat	ggg	caa	caa	gtc	ctt	tgc	1505
Thr	Val	Phe	Val	Glu	Ala	Asn	Ile	Gln	His	Gly	Gln	Gln	Val	Leu	Cys	
	380					385					390					
caa	agg	aga	acc	agc	ccc	aaa	ccc	ttc	aca	gag	gag	gtg	ctg	tgg	aat	1553
Gln	Arg	Arg	Thr	Ser	Pro	Lys	Pro	Phe	Thr	Glu	Glu	Val	Leu	Trp	Asn	
395					400					405					410	
gtg	tgg	ctt	gag	ttc	agt	atc	aaa	atc	aaa	gac	ttg	ccc	aaa	ggg	gct	1601
Val	Trp	Leu	Glu	Phe	Ser	Ile	Lys	Ile	Lys	Asp	Leu	Pro	Lys	Gly	Ala	
				415					420					425		
cta	ctg	aac	ctc	cag	atc	tac	tgc	ggg	aaa	gct	cca	gca	ctg	tcc	agc	1649
Leu	Leu	Asn	Leu	Gln	Ile	Tyr	Cys	Gly	Lys	Ala	Pro	Ala	Leu	Ser	Ser	
			430				435						440			
aag	gcc	tct	gca	gag	tcc	ccc	agt	tct	gag	tcc	aag	ggc	aaa	gtt	cgg	1697
Lys	Ala	Ser	Ala	Glu	Ser	Pro	Ser	Ser	Glu	Ser	Lys	Gly	Lys	Val	Arg	
		445				450						455				
ctt	ctc	tat	tat	gtg	aac	ctg	ctg	ctg	ata	gac	cac	cgt	ttc	ctc	ctg	1745
Leu	Leu	Tyr	Tyr	Val	Asn	Leu	Leu	Leu	Ile	Asp	His	Arg	Phe	Leu	Leu	
	460				465						470					
cgc	cgt	gga	gaa	tac	gtc	ctc	cac	atg	tgg	cag	ata	tct	ggg	aag	gga	1793
Arg	Arg	Gly	Glu	Tyr	Val	Leu	His	Met	Trp	Gln	Ile	Ser	Gly	Lys	Gly	
475					480					485					490	
gaa	gac	caa	gga	agc	ttc	aat	gct	gac	aaa	ctc	acg	tct	gca	act	aac	1841
Glu	Asp	Gln	Gly	Ser	Phe	Asn	Ala	Asp	Lys	Leu	Thr	Ser	Ala	Thr	Asn	
				495					500					505		

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cca gac aag gag aac tca atg tcc atc tcc att ctt ctg gac aat tac	1889
Pro Asp Lys Glu Asn Ser Met Ser Ile Ser Ile Leu Leu Asp Asn Tyr	
510 515 520	
tgc cac ccg ata gcc ctg cct aag cat cag ccc acc cct gac ccg gaa	1937
Cys His Pro Ile Ala Leu Pro Lys His Gln Pro Thr Pro Asp Pro Glu	
525 530 535	
ggg gac ccg gtt cga gca gaa atg ccc aac cag ctt cgc aag caa ttg	1985
Gly Asp Arg Val Arg Ala Glu Met Pro Asn Gln Leu Arg Lys Gln Leu	
540 545 550	
gag gcg atc ata gcc act gat cca ctt aac cct ctc aca gca gag gac	2033
Glu Ala Ile Ile Ala Thr Asp Pro Leu Asn Pro Leu Thr Ala Glu Asp	
555 560 565 570	
aaa gaa ttg ctc tgg cat ttt aga tac gaa agc ctt aag cac cca aaa	2081
Lys Glu Leu Leu Trp His Phe Arg Tyr Glu Ser Leu Lys His Pro Lys	
575 580 585	
gca tat cct aag cta ttt agt tca gtg aaa tgg gga cag caa gaa att	2129
Ala Tyr Pro Lys Leu Phe Ser Ser Val Lys Trp Gly Gln Gln Glu Ile	
590 595 600	
gtg gcc aaa aca tac caa ttg ttg gcc aga agg gaa gtc tgg gat caa	2177
Val Ala Lys Thr Tyr Gln Leu Leu Ala Arg Arg Glu Val Trp Asp Gln	
605 610 615	
agt gct ttg gat gtt ggg tta aca atg cag ctc ctg gac tgc aac ttc	2225
Ser Ala Leu Asp Val Gly Leu Thr Met Gln Leu Leu Asp Cys Asn Phe	
620 625 630	
tca gat gaa aat gta aga gcc att gca gtt cag aaa ctg gag agc ttg	2273
Ser Asp Glu Asn Val Arg Ala Ile Ala Val Gln Lys Leu Glu Ser Leu	
635 640 645 650	
gag gac gat gat gtt ctg cat tac ctt cta caa ttg gtc cag gct gtg	2321
Glu Asp Asp Asp Val Leu His Tyr Leu Leu Gln Leu Val Gln Ala Val	
655 660 665	
aaa ttt gaa cca tac cat gat agc gcc ctt gcc aga ttt ctg ctg aag	2369
Lys Phe Glu Pro Tyr His Asp Ser Ala Leu Ala Arg Phe Leu Leu Lys	
670 675 680	
cgt ggt tta aga aac aaa aga att ggt cac ttt ttg ttt tgg ttc ttg	2417
Arg Gly Leu Arg Asn Lys Arg Ile Gly His Phe Leu Phe Trp Phe Leu	
685 690 695	
aga agt gag ata gcc cag tcc aga cac tat cag cag agg ttc gct gtg	2465
Arg Ser Glu Ile Ala Gln Ser Arg His Tyr Gln Gln Arg Phe Ala Val	
700 705 710	
att ctg gaa gcc tat ctg agg ggc tgt ggc aca gcc atg ctg cac gac	2513
Ile Leu Glu Ala Tyr Leu Arg Gly Cys Gly Thr Ala Met Leu His Asp	
715 720 725 730	
ttt acc caa caa gtc caa gta atc gag atg tta caa aaa gtc acc ctt	2561
Phe Thr Gln Gln Val Gln Val Ile Glu Met Leu Gln Lys Val Thr Leu	
735 740 745	

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gat att aaa tcg ctc tct gct gaa aag tat gac gtc agt tcc caa gtt	2609
Asp Ile Lys Ser Leu Ser Ala Glu Lys Tyr Asp Val Ser Ser Gln Val	
750 755 760	
att tca caa ctt aaa caa aag ctt gaa aac ctg cag aat tct caa ctc	2657
Ile Ser Gln Leu Lys Gln Lys Leu Glu Asn Leu Gln Asn Ser Gln Leu	
765 770 775	
ccc gaa agc ttt aga gtt cca tat gat cct gga ctg aaa gca gga gcg	2705
Pro Glu Ser Phe Arg Val Pro Tyr Asp Pro Gly Leu Lys Ala Gly Ala	
780 785 790	
ctg gca att gaa aaa tgt aaa gta atg gcc tcc aag aaa aaa cca cta	2753
Leu Ala Ile Glu Lys Cys Lys Val Met Ala Ser Lys Lys Lys Pro Leu	
795 800 805 810	
tgg ctt gag ttt aaa tgt gcc gat cct aca gcc cta tca aat gaa aca	2801
Trp Leu Glu Phe Lys Cys Ala Asp Pro Thr Ala Leu Ser Asn Glu Thr	
815 820 825	
att gga att atc ttt aaa cat ggt gat gat ctg cgc caa gac atg ctt	2849
Ile Gly Ile Ile Phe Lys His Gly Asp Asp Leu Arg Gln Asp Met Leu	
830 835 840	
att tta cag att cta cga atc atg gag tct att tgg gag act gaa tct	2897
Ile Leu Gln Ile Leu Arg Ile Met Glu Ser Ile Trp Glu Thr Glu Ser	
845 850 855	
ttg gat cta tgc ctc ctg cca tat ggt tgc att tca act ggt gac aaa	2945
Leu Asp Leu Cys Leu Leu Pro Tyr Gly Cys Ile Ser Thr Gly Asp Lys	
860 865 870	
ata gga atg atc gag att gtg aaa gac gcc acg aca att gcc aaa att	2993
Ile Gly Met Ile Glu Ile Val Lys Asp Ala Thr Thr Ile Ala Lys Ile	
875 880 885 890	
cag caa agc aca gtg ggc aac acg gga gca ttt aaa gat gaa gtc ctg	3041
Gln Gln Ser Thr Val Gly Asn Thr Gly Ala Phe Lys Asp Glu Val Leu	
895 900 905	
aat cac tgg ctc aaa gaa aaa tcc cct act gaa gaa aag ttt cag gca	3089
Asn His Trp Leu Lys Glu Lys Ser Pro Thr Glu Glu Lys Phe Gln Ala	
910 915 920	
gca gtg gag aga ttt gtt tat tcc tgt gca ggc tac tgt gtg gca acc	3137
Ala Val Glu Arg Phe Val Tyr Ser Cys Ala Gly Tyr Cys Val Ala Thr	
925 930 935	
ttt gtt ctt gga ata ggc gac aga cac aat gac aat att atg atc acc	3185
Phe Val Leu Gly Ile Gly Asp Arg His Asn Asp Asn Ile Met Ile Thr	
940 945 950	
gag aca gga aac cta ttt cat att gac ttc ggg cac att ctt ggg aat	3233
Glu Thr Gly Asn Leu Phe His Ile Asp Phe Gly His Ile Leu Gly Asn	
955 960 965 970	
tac aaa agt ttc ctg ggc att aat aaa gag aga gtg cca ttt gtg cta	3281
Tyr Lys Ser Phe Leu Gly Ile Asn Lys Glu Arg Val Pro Phe Val Leu	
975 980 985	

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acc cct gac ttc ctc ttt gtg atg gga act tct gga aag aag aca agc 3329  
 Thr Pro Asp Phe Leu Phe Val Met Gly Thr Ser Gly Lys Lys Thr Ser  
 990 995 1000

cca cac ttc cag aaa ttt cag gac atc tgt gtt aag gct tat cta gcc 3377  
 Pro His Phe Gln Lys Phe Gln Asp Ile Cys Val Lys Ala Tyr Leu Ala  
 1005 1010 1015

ctt cgt cat cac aca aac cta ctg atc atc ctg ttc tcc atg atg ctg 3425  
 Leu Arg His His Thr Asn Leu Leu Ile Ile Leu Phe Ser Met Met Leu  
 1020 1025 1030

atg aca gga atg ccc cag tta aca agc aaa gaa gac att gaa tat atc 3473  
 Met Thr Gly Met Pro Gln Leu Thr Ser Lys Glu Asp Ile Glu Tyr Ile  
 1035 1040 1045 1050

cgg gat gcc ctc aca gtg ggg aaa aat gag gag gat gct aaa aag tat 3521  
 Arg Asp Ala Leu Thr Val Gly Lys Asn Glu Glu Asp Ala Lys Lys Tyr  
 1055 1060 1065

ttt ctt gat cag atc gaa gtt tgc aga gac aaa gga tgg act gtg cag 3569  
 Phe Leu Asp Gln Ile Glu Val Cys Arg Asp Lys Gly Trp Thr Val Gln  
 1070 1075 1080

ttt aat tgg ttt cta cat ctt gtt ctt ggc atc aaa caa gga gag aaa 3617  
 Phe Asn Trp Phe Leu His Leu Val Leu Gly Ile Lys Gln Gly Glu Lys  
 1085 1090 1095

cat tca gcc taa tacttttaggc tagaatcaaa aacaagttag tggttctatgg 3669  
 His Ser Ala  
 1100

tttaaattag catagcaatc atcgaacttg gatttcaaat gcaatagaca ttgtgaaagc 3729  
 tggcatttca gaagtatagc tcttttcccta cctgaactct tccctggaga aaagatgttg 3789  
 gcattgctga ttgtttgggt aagcaatgtc cagtgttagg attatttgca gggttggttt 3849  
 tttctcatth gtctgtggca ttggagaata ttctcggttt aaacagacta atgacttcct 3909  
 tattgtccct gatattttga ctatcttact attgagtgtc tctggaaatt ctttggaata 3969  
 attgatgaca tctattttca tctgggttta gtctcaattt tgggtatctt tgtgttcctc 4029  
 aagctcttta aagaaaaaga tgtaatcggt gtaacctttg tctcattcct taaatgatgc 4089  
 ttccaaacat ctcttagtg tctgcagggt ttagtggtgt gctaaaagca aggaaagcga 4149  
 gttagtcttt tcagtgtctt ttgcaattca attcttttgt catgtataac tgagacacac 4209  
 aaacacagca ggagaaatct aaaccgttgt gccttgacct tcctctgctg gtcttggtcc 4269  
 agggttatga atatgaaaaa atagagatga gactttttgt gtcaactctg tccacaagag 4329  
 tgagttatct agtatgatta gtatagcttt ctccagcatg gcagcaggaa gtaactacag 4389  
 ggctcttttt atgcctgaca tttcttccct tcctttttcc ctgcctccct ttttcatcaa 4449  
 ttgcaatgct cccacaactc ttacagact tgtgaaatct tcaagaacac ctttactcta 4509  
 taactcaaaa attagttgaa aaataattac ttctcaagga ttattagaat cttaggtact 4569



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tatttgtaaa gatgttttagt gacttttttt tcaagtatct ataaaggagg cagattcttag 4629  
 aaaatatgaa ttagttttcca aatgccttaa ttttaaactt tggcctgaac agtttttttct 4689  
 ttttcttaat ggaagaagat atttaatatc ttaaaaaatat tccaagttag gaagaacact 4749  
 acttgcccta tccattttccc atttaaagga ctttttaaact ttgacacagt ctttcagatt 4809  
 tcctgaaaat ctttgaaata tcttacttta aaaatatattt catctctgaa atatctcggt 4869  
 atttattgga ggtattggtt aaccttagat agaccattaa attatttata aaatattttg 4929  
 taattactgt agctaataca ttacatagaa aaaactatgt taacagtgtc tctgtttaag 4989  
 tataatcaga tataaatata taacttaatt ttttaatttt aaaaaataga tacctggttg 5049  
 actttgaggt agtccaggcc tttttctttt tttttttttt taatgtgtgc aaaagcccaa 5109  
 aggttcctaa gcctggctgc aaagaagaat caacagggac acttttttaa aacactctta 5169  
 tcagcctggg gcaacacagt gagactccat ctcttaaaaa aaaaattagc tgggtatagt 5229  
 ggtatgtgcc tgtagtccca ggtactcagg aggctgagggc aggaggattg cctgagccca 5289  
 ggaggtggaa actgcagaga gtcgatgaca tgtccttaca ctccagcctg gataacagag 5349  
 cgagaccctg tctcaaaaaa aaaaaaaaaa aaaaaaaaaa aactcgag 5397

&lt;210&gt; 10

&lt;211&gt; 1101

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 10

Met Glu Leu Glu Asn Tyr Lys Gln Pro Val Val Leu Arg Glu Asp Asn  
 1 5 10 15

Cys Arg Arg Arg Arg Arg Met Lys Pro Arg Ser Ala Ala Ser Leu Ser  
 20 25 30

Ser Met Glu Leu Ile Pro Ile Glu Phe Val Leu Pro Thr Ser Gln Arg  
 35 40 45

Lys Cys Lys Ser Pro Glu Thr Ala Leu Leu His Val Ala Gly His Gly  
 50 55 60

Asn Val Glu Gln Met Lys Ala Gln Val Trp Leu Arg Ala Leu Glu Thr  
 65 70 75 80

Ser Val Ala Ala Asp Phe Tyr His Arg Leu Gly Pro His His Phe Leu  
 85 90 95

Leu Leu Tyr Gln Lys Lys Gly Gln Trp Tyr Glu Ile Tyr Asp Lys Tyr  
 100 105 110

Gln Val Val Gln Thr Leu Asp Cys Leu Arg Tyr Trp Lys Ala Thr His  
 115 120 125

Arg Ser Pro Gly Gln Ile His Leu Val Gln Arg His Pro Pro Ser Glu  
 130 135 140

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Glu Ser Gln Ala Phe Gln Arg Gln Leu Thr Ala Leu Ile Gly Tyr Asp  
 145 150 155 160  
 Val Thr Asp Val Ser Asn Val His Asp Asp Glu Leu Glu Phe Thr Arg  
 165 170 175  
 Arg Gly Leu Val Thr Pro Arg Met Ala Glu Val Ala Ser Arg Asp Pro  
 180 185 190  
 Lys Leu Tyr Ala Met His Pro Trp Val Thr Ser Lys Pro Leu Pro Glu  
 195 200 205  
 Tyr Leu Trp Lys Lys Ile Ala Asn Asn Cys Ile Phe Ile Val Ile His  
 210 215 220  
 Arg Ser Thr Thr Ser Gln Thr Ile Lys Val Ser Pro Asp Asp Thr Pro  
 225 230 235 240  
 Gly Ala Ile Leu Gln Ser Phe Phe Thr Lys Met Ala Lys Lys Lys Ser  
 245 250 255  
 Leu Met Asp Ile Pro Glu Ser Gln Ser Glu Gln Asp Phe Val Leu Arg  
 260 265 270  
 Val Cys Gly Arg Asp Glu Tyr Leu Val Gly Glu Thr Pro Ile Lys Asn  
 275 280 285  
 Phe Gln Trp Val Arg His Cys Leu Lys Asn Gly Glu Glu Ile His Val  
 290 295 300  
 Val Leu Asp Thr Pro Pro Asp Pro Ala Leu Asp Glu Val Arg Lys Glu  
 305 310 315 320  
 Glu Trp Pro Leu Val Asp Asp Cys Thr Gly Val Thr Gly Tyr His Glu  
 325 330 335  
 Gln Leu Thr Ile His Gly Lys Asp His Glu Ser Val Phe Thr Val Ser  
 340 345 350  
 Leu Trp Asp Cys Asp Arg Lys Phe Arg Val Lys Ile Arg Gly Ile Asp  
 355 360 365  
 Ile Pro Val Leu Pro Arg Asn Thr Asp Leu Thr Val Phe Val Glu Ala  
 370 375 380  
 Asn Ile Gln His Gly Gln Gln Val Leu Cys Gln Arg Arg Thr Ser Pro  
 385 390 395 400  
 Lys Pro Phe Thr Glu Glu Val Leu Trp Asn Val Trp Leu Glu Phe Ser  
 405 410 415  
 Ile Lys Ile Lys Asp Leu Pro Lys Gly Ala Leu Leu Asn Leu Gln Ile  
 420 425 430  
 Tyr Cys Gly Lys Ala Pro Ala Leu Ser Ser Lys Ala Ser Ala Glu Ser  
 435 440 445  
 Pro Ser Ser Glu Ser Lys Gly Lys Val Arg Leu Leu Tyr Tyr Val Asn  
 450 455 460  
 Leu Leu Leu Ile Asp His Arg Phe Leu Leu Arg Arg Gly Glu Tyr Val  
 465 470 475 480

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Leu	His	Met	Trp	Gln	Ile	Ser	Gly	Lys	Gly	Glu	Asp	Gln	Gly	Ser	Phe	485	490	495
Asn	Ala	Asp	Lys	Leu	Thr	Ser	Ala	Thr	Asn	Pro	Asp	Lys	Glu	Asn	Ser	500	505	510
Met	Ser	Ile	Ser	Ile	Leu	Leu	Asp	Asn	Tyr	Cys	His	Pro	Ile	Ala	Leu	515	520	525
Pro	Lys	His	Gln	Pro	Thr	Pro	Asp	Pro	Glu	Gly	Asp	Arg	Val	Arg	Ala	530	535	540
Glu	Met	Pro	Asn	Gln	Leu	Arg	Lys	Gln	Leu	Glu	Ala	Ile	Ile	Ala	Thr	545	550	555
Asp	Pro	Leu	Asn	Pro	Leu	Thr	Ala	Glu	Asp	Lys	Glu	Leu	Leu	Trp	His	565	570	575
Phe	Arg	Tyr	Glu	Ser	Leu	Lys	His	Pro	Lys	Ala	Tyr	Pro	Lys	Leu	Phe	580	585	590
Ser	Ser	Val	Lys	Trp	Gly	Gln	Gln	Glu	Ile	Val	Ala	Lys	Thr	Tyr	Gln	595	600	605
Leu	Leu	Ala	Arg	Arg	Glu	Val	Trp	Asp	Gln	Ser	Ala	Leu	Asp	Val	Gly	610	615	620
Leu	Thr	Met	Gln	Leu	Leu	Asp	Cys	Asn	Phe	Ser	Asp	Glu	Asn	Val	Arg	625	630	635
Ala	Ile	Ala	Val	Gln	Lys	Leu	Glu	Ser	Leu	Glu	Asp	Asp	Asp	Val	Leu	645	650	655
His	Tyr	Leu	Leu	Gln	Leu	Val	Gln	Ala	Val	Lys	Phe	Glu	Pro	Tyr	His	660	665	670
Asp	Ser	Ala	Leu	Ala	Arg	Phe	Leu	Leu	Lys	Arg	Gly	Leu	Arg	Asn	Lys	675	680	685
Arg	Ile	Gly	His	Phe	Leu	Phe	Trp	Phe	Leu	Arg	Ser	Glu	Ile	Ala	Gln	690	695	700
Ser	Arg	His	Tyr	Gln	Gln	Arg	Phe	Ala	Val	Ile	Leu	Glu	Ala	Tyr	Leu	705	710	715
Arg	Gly	Cys	Gly	Thr	Ala	Met	Leu	His	Asp	Phe	Thr	Gln	Gln	Val	Gln	725	730	735
Val	Ile	Glu	Met	Leu	Gln	Lys	Val	Thr	Leu	Asp	Ile	Lys	Ser	Leu	Ser	740	745	750
Ala	Glu	Lys	Tyr	Asp	Val	Ser	Ser	Gln	Val	Ile	Ser	Gln	Leu	Lys	Gln	755	760	765
Lys	Leu	Glu	Asn	Leu	Gln	Asn	Ser	Gln	Leu	Pro	Glu	Ser	Phe	Arg	Val	770	775	780
Pro	Tyr	Asp	Pro	Gly	Leu	Lys	Ala	Gly	Ala	Leu	Ala	Ile	Glu	Lys	Cys	785	790	795
																		800

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Lys Val Met Ala Ser Lys Lys Lys Pro Leu Trp Leu Glu Phe Lys Cys  
 805 810 815  
 Ala Asp Pro Thr Ala Leu Ser Asn Glu Thr Ile Gly Ile Ile Phe Lys  
 820 825 830  
 His Gly Asp Asp Leu Arg Gln Asp Met Leu Ile Leu Gln Ile Leu Arg  
 835 840 845  
 Ile Met Glu Ser Ile Trp Glu Thr Glu Ser Leu Asp Leu Cys Leu Leu  
 850 855 860  
 Pro Tyr Gly Cys Ile Ser Thr Gly Asp Lys Ile Gly Met Ile Glu Ile  
 865 870 875 880  
 Val Lys Asp Ala Thr Thr Ile Ala Lys Ile Gln Gln Ser Thr Val Gly  
 885 890 895  
 Asn Thr Gly Ala Phe Lys Asp Glu Val Leu Asn His Trp Leu Lys Glu  
 900 905 910  
 Lys Ser Pro Thr Glu Glu Lys Phe Gln Ala Ala Val Glu Arg Phe Val  
 915 920 925  
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 His Ile Asp Phe Gly His Ile Leu Gly Asn Tyr Lys Ser Phe Leu Gly  
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 995 1000 1005  
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Glu Asp Glu Val Ser Phe Gln Asp Gly Asp Thr Ile Val Asn Val Gln
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Gln Ile Asp Asp Gly Trp Met Tyr Gly Thr Val Glu Arg Thr Gly Asp
      30             35             40             45

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Thr Gly Met Leu Pro Ala Asn Tyr Val Glu Ala Ile
              50             55

gcgcgcccgcc cctgtcttca atgcattcca tggcatcaca tctgtcctgg ggccctgaccc 251
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Met Asn Pro Asn Cys Ala Arg Cys Gly Lys Ile Val Tyr Pro Thr
      1             5             10             15

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-41-

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tgc gag acc tgc aag atg acc ctg aac atg aag aac tac aag ggt tat	205
Cys Glu Thr Cys Lys Met Thr Leu Asn Met Lys Asn Tyr Lys Gly Tyr	
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Gln Pro Gln Pro His His Ile Pro Thr Ser Ala Pro Val Tyr Gln Gln	
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ccc cag cag cag cag atg acc tcg tcc tat ggt ggg tac aag gag cca	637
Pro Gln Gln Gln Gln Met Thr Ser Ser Tyr Gly Gly Tyr Lys Glu Pro	
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gca gcc cct gtc tcc ata cag cgc agt gcc cca ggt ggc ggt ggg aaa	685
Ala Ala Pro Val Ser Ile Gln Arg Ser Ala Pro Gly Gly Gly Gly Lys	
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225 230 235	
ggc tgg atg tac ggg acc gta gag cgc acc ggt gac acg ggg atg ctg	829
Gly Trp Met Tyr Gly Thr Val Glu Arg Thr Gly Asp Thr Gly Met Leu	
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-42-

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 50 55 60  
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 Gly Phe Ser Val Ala Asp Thr Pro Glu Leu Gln Arg Ile Lys Lys  
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 Thr Gln Asp Gln Ile Ser Asn Ile Lys Tyr His Glu Glu Phe Glu Lys  
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 130 135 140  
 Glu Ala Gln Asp Ser Ser Ser Tyr Arg Arg Pro Thr Glu Gln Gln Gln  
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 180 185 190  
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 195 200 205  
 Tyr Arg Ala Val Tyr Asp Tyr Ser Ala Ala Asp Glu Asp Glu Val Ser  
 210 215 220  
 Phe Gln Asp Gly Asp Thr Ile Val Asn Val Gln Gln Ile Asp Asp Gly  
 225 230 235 240  
 Trp Met Tyr Gly Thr Val Glu Arg Thr Gly Asp Thr Gly Met Leu Pro  
 245 250 255



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Ala Asn Tyr Val Glu Ala Ile  
260

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(21) International Application Number: PCT/US01/15065

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(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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Published:

— with international search report

(88) Date of publication of the international search report:  
27 December 2002

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*



**WO 01/085986 A3**

(54) Title: PHOSPHATIDYL INOSITOL 3-KINASE DELTA BINDING PARTNER

(57) Abstract: There is identified a functional interaction between the catalytic subunit of phosphatidyl inositol 3-kinase delta (P13K $\delta$ ) and SH3 domain-containing polypeptides such as LASP-1. The invention provides methods of assaying the observed interaction, methods of exploiting the interaction to identify compounds that modulate the interaction, and methods of employing such modulators in the treatment of medical disorders characterized by P13K $\delta$  activity mediated by the interaction.

## INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 01/15065

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12Q1/48 A61P19/00 A61P29/00 A61P35/00 A61P37/00  
G01N33/50 C12N9/12

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 G01N C12Q C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, MEDLINE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 985 589 A (CHANTRY DAVID H ET AL) 16 November 1999 (1999-11-16) cited in the application abstract claims 2,3 column 4, line 25 - line 42	19-35
Y	---	36,37
	--- -/--	

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
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Date of the actual completion of the international search

21 June 2002

Date of mailing of the international search report

10/10/2002

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Stricker, J-E

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/15065

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>DIDICHENKO S A ET AL: "CONSTITUTIVE ACTIVATION OF PROTEIN KINASE B AND PHOSPHORYLATION OF P47PHOX BY A MEMBRANE-TARGETED PHOSPHOINOSITIDE 3-KINASE"</p> <p>CURRENT BIOLOGY, CURRENT SCIENCE,, GB, vol. 6, no. 10, 1 October 1996 (1996-10-01), pages 1271-1278, XP002037800</p> <p>ISSN: 0960-9822</p> <p>abstract</p> <p>---</p>	36
Y	<p>DATABASE BIOSIS 'Online!</p> <p>BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US;</p> <p>31 March 1998 (1998-03-31)</p> <p>QIU YUN ET AL: "Etk/Bmx, a tyrosine kinase with a pleckstrin-homology domain, is an effector of phosphatidylinositol 3'-kinase and is involved in interleukin 6-induced neuroendocrine differentiation of prostate cancer cells."</p> <p>Database accession no. PREV199800230700</p> <p>XP002203057</p> <p>abstract</p> <p>&amp; PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 95, no. 7, 31 March 1998 (1998-03-31), pages 3644-3649, March 31, 1998</p> <p>ISSN: 0027-8424</p> <p>---</p>	37
A	<p>CHANTRY D ET AL: "P110DELTA, A NOVEL PHOSPHATIDYLINOSITOL 3-KINASE CATALYTIC SUBUNIT THAT ASSOCIATES WITH P85 AND IS EXPRESSED PREDOMINANTLY IN LEUKOCYTES"</p> <p>JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 272, no. 31, 1 August 1997 (1997-08-01), pages 19236-19241, XP002059998</p> <p>ISSN: 0021-9258</p> <p>cited in the application</p> <p>abstract</p> <p>---</p> <p>-/--</p>	19-35

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/15065

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>VANHAESEBROECK B ET AL: "P110BETA, A NOVEL PHOSPHOINOSITIDE 3-KINASE IN LEUKOCYTES"</p> <p>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 94, no. 9, 29 April 1997 (1997-04-29), pages 4330-4335, XP002044005 ISSN: 0027-8424 cited in the application abstract</p> <p>---</p>	19-35
A	<p>DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; July 1998 (1998-07)</p> <p>CHEW C S ET AL: "Lasp-1 is a regulated phosphoprotein within the cAMP signaling pathway in the gastric parietal cell." Database accession no. PREV199800388860 XP002202774 cited in the application abstract</p> <p>&amp; AMERICAN JOURNAL OF PHYSIOLOGY, vol. 275, no. 1 PART1, July 1998 (1998-07), pages C56-C67, ISSN: 0002-9513</p> <p>---</p>	1-18
A	<p>KLIPPEL A ET AL: "A REGION OF THE 85-KILODALTON (KDA) SUBUNIT OF PHOSPHATIDYLINOSITOL 3-KINASE BINDS THE 110-KDA CATALYTIC SUBUNIT IN VIVO"</p> <p>MOLECULAR AND CELLULAR BIOLOGY, WASHINGTON, DC, US, vol. 13, no. 9, 1 September 1993 (1993-09-01), pages 5560-5566, XP002037804 ISSN: 0270-7306 abstract</p> <p>-----</p>	

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/01/15065

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 13-18, 29-37  
because they relate to subject matter not required to be searched by this Authority, namely:  
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☒ Claims Nos.: 19-34 (partially), 11, 12, 27 and 28 (totally)  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:  
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

## Continuation of Box I.1

Although claims 13-18 and 29-37 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

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## Continuation of Box I.1

Claims Nos.: 13-18, 29-37

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

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## Continuation of Box I.2

Claims Nos.: 19-34 (partially), 11, 12, 27 and 28 (totally)

Present claims 19-34 relate to an extremely large number of possible polypeptides comprising an SH3 domain. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found (p.36, 1.10), however, for only a reduced number of polypeptides. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the polypeptides p85, p47-phox and Btk.

Present claims 11, 12, 27 and 28 relate to a substance and its use defined by reference to a desirable characteristic or property, namely an activator or inhibitor of the binding between p110 delta and an SH3 containing polypeptide, e.g. LASP-1.

The said claims cover all substances having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such substances. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the substances by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international



## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

# INTERNATIONAL SEARCH REPORT

Info on patent family members

International Application No

PCT/US 01/15065

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 5985589	A	16-11-1999	US 5882910 A	16-03-1999
			US 5858753 A	12-01-1999
			AU 5458798 A	22-06-1998
			CA 2243944 A1	04-06-1998
			EP 0891428 A1	20-01-1999
			JP 2000505653 T	16-05-2000
			WO 9823760 A1	04-06-1998

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